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Bioefficacy of native *Bacillus thuringiensis* isolates from the Western Ghats of Kerala on pumpkin caterpillar, *Diaphania indica* (Saund.) (Lepidoptera: Pyralidae)

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ABSTRACT: Bioefficacy of crude protein from eleven *Bacillus thuringiensis* isolates obtained from the Western Ghats region of Kerala was tested by diet contamination method, on second instar larvae of pumpkin caterpillar, *Diaphania indica* (Saund), a major lepidopteran pest of cucurbitaceous vegetables in Kerala. Based on statistical analysis of per cent mortality of larvae, the isolates KK7, KK8, KK9 and KY2 were on par, but significantly less efficient than the reference strain HD1. Profiling of *cry*1 and *cry*4 genes from *B. thuringiensis* isolates was done using universal *cry*1 and *cry*4 primers for detection of *cry* genes and prediction of their insecticidal activities. Amplification with universal *cry*1 gene primer was obtained for eight isolates KY2, KY3, KY5, KY6, KK7, KY8, KY9 and EM11 along with HD1. Amplification for *cry*4 gene was obtained for isolates KY1 and EM10 but not *cry*1 gene. KY4 did not show *cry*1 or *cry*4 gene though it was also toxic to *D. indica*. New genes, unidentified in the present investigation, are involved in the toxicity of isolates without *cry*1 gene.

KEYWORDS: *Bacillus thuringiensis*, *Diaphania indica*, bioefficacy, crystal proteins, *cry* gene

INTRODUCTION

The crystalliferous bacterium, *Bacillus thuringiensis* Berliner is the most widely used environment friendly alternative to chemical pesticides for the biological control of pests of agricultural crops and forest trees (Lambert, 1992). Research efforts to improve formulations based on the bacterium to increase the toxicity spectrum or to understand the mechanism of action of toxins produced by the bacilli heavily rely on bioassay against target pests. The development of transgenic plants expressing the

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B. thuringiensis toxin and their significant use in integrated pest management created interest for new isolates of this micro organism against lepidopteran pests. Moreover, there is an increasing concern over development of resistance when a single gene is used in transgenics. Western Ghats is one among the 18 biodiversity hotspots in the World and is least explored for microbial diversity. The present investigations were directed at exploring the bioefficacy of native *B. thuringiensis* isolates from the Western Ghats of Kerala against a serious lepidopteran pest of cucurbitaceous vegetables, the pumpkin caterpillar, *Diaphania indica* (Saund.).

MATERIALS AND METHODS

Laboratory experiments were conducted to assess the bioefficacy of *B. thuringiensis* isolates against *D. indica*. Eleven bacterial isolates were obtained from soils of the Western Ghats from the districts of Kottayam, Kozhikode and Ernakulam, by Travers' method (Travers *et al.*, 1987). These isolates along with the reference strain HD1 were stored as stabs in cryostorage vials in LB agar medium under refrigerated condition.

Crude preparation of crystal protein was extracted from *B. thuringiensis* (Dulmage, 1970). The toxicity of crystal protein was tested on four- to five day old larvae of *D. indica* by diet contamination method, as described by Schesser *et al.* (1977).

Larval mortality was recorded at an interval of 24 h, till all the larvae died/pupated and the per cent mortality was corrected using Abbot's formula.

Statistical analysis was done using Kendall's coefficient of concordance by SPSS software, a statistical package (Kendall and Smith, 1939). The isolates were ranked on the basis of mortality on fifth and eighth days.

Screening of isolates for the presence of *cry*1 and *cry*4 genes was done using universal primers designed by Ben-Dov *et al.* (1997). The total DNA of *B. thuringiensis* isolates and reference strains used for PCR amplification was isolated following the procedure of Sambrook and Russel (2001). A set of universal primers Un1(d), Un1(r), Un4(d) and Un4(r) (Ben-Dov *et al.*, 1997) were used for the amplification of *cry*1 and *cry*4 genes. The primers were custom synthesized by Integrated DNA Technologies, USA. Other components of PCR reaction mix were obtained from Bangalore Genei. PCR was carried out by standard procedure (Sambrook and Russel, 2001) in Eppendorf Master Cycler, Gradient (Eppendorf, Germany) under the following conditions: 2 min of initial denaturation at 94 °C, followed by 30 cycles of denaturation for 45 seconds at 94 °C, annealing for 1 min at 55 °C depending on DNA template and extension for 2 min at 72 °C. A final extension of 10 min at 72 °C was given after completion of 35 cycles. PCR products were resolved on 0.8 per cent agarose gel stained with ethidium bromide and λ DNA/*Eco* R1+ *Hind* III double digest as a DNA molecular weight marker. The DNA bands in gels were documented using gel documentation system (Alpha imager TM1200).

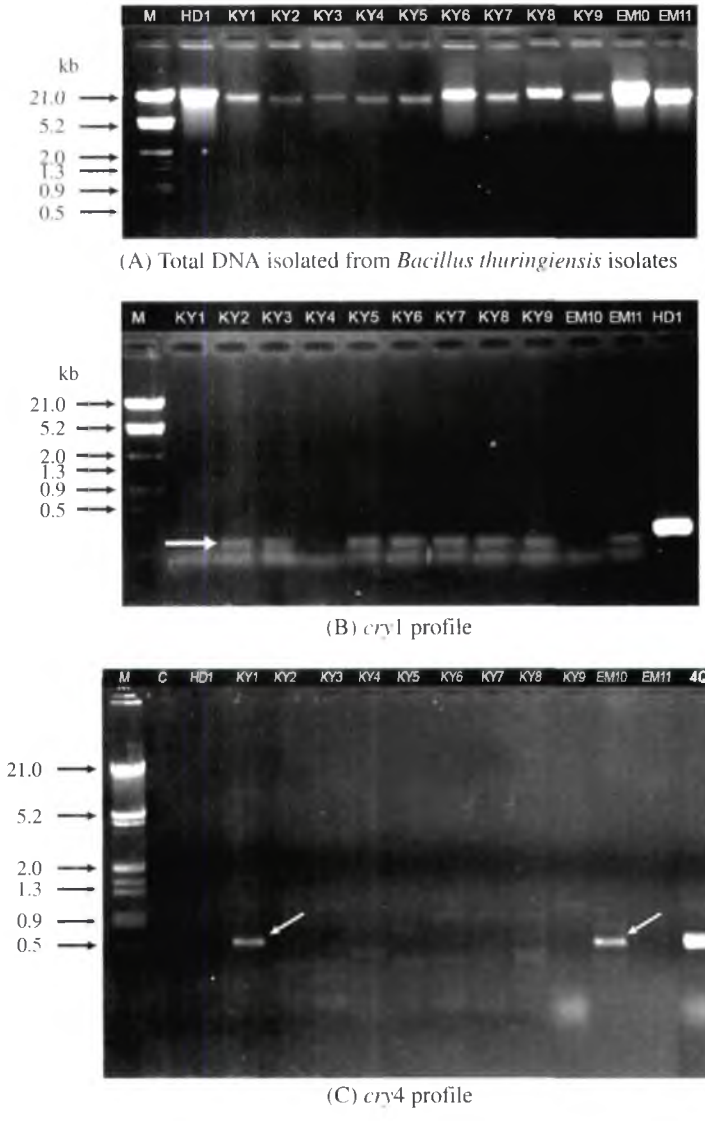
TABLE 1. Mortality and mean rank score of crystal proteins of *Bacillus thuringiensis* isolates on *Diaphania indica* larvae

Isolate	5th day		8th day	
	Per cent Mortality	Mean Rank Score	Per cent mortality	Mean Rank Score
KY1	57.7	6.13	94.4	4.64
KY2	70.1	8.50	84.4	7.57
KY3	65.1	2.88	100	5.21
KY4	70.1	1.50	94.4	3.57
KY5	70.1	7.00	100	6.36
KY6	62.6	6.38	94.4	6.00
KK7	85	8.75	95	9.14
KK8	85	8.50	100	9.29
KK9	70.2	8.50	89.2	8.14
EM10	50.4	6.00	88.8	4.34
EM11	38.3	2.13	76.1	1.64
HD1	100	11.75	100	12.50

RESULTS

The mortality response of *D. indica* larvae to *B. thuringiensis* crystal proteins revealed that the reference strain HD1 caused 100% mortality by fifth day. Among the native isolates, highest mortality was obtained for the isolate KK7 and KK8, with 85% mortality on fifth day and 85% and 100% mortality on 8th day. This was followed by the isolates KK9, KY2, KY4 and KY5 with about 70% mortality on 5th day and 84–100% mortality on 8th day (Table 1). HD1 showed the highest rank scores (11.75 and 12.50 on 5th and 8th days, respectively). Isolate KK7 proved to be a potential isolate with a mean score of 8.75. Isolates KK8, KK9 and KY2 had the same efficacy with a mean rank score of 8.5.

Amplification was obtained for *cry*1 gene primer, at an annealing temperature of 55 °C. Amplification of a single band of less than 250 bp was obtained with universal *cry*1 gene primer for eight isolates KY2, KY3, KY5, KY6, KK7 KK8, KK9 and EM11 and strain HD1 (Fig. 1, Table 2). No amplification was obtained for isolates KY1, KY4 and EM10. Screening of *B. thuringiensis* isolates was carried out for *cry*4 gene with universal *cry*4 primer (Ben-Dov *et al.*, 1997). Amplification for *cry*4 gene was obtained for reference strain 4Q1 and native isolates KY1 and EM10. Single band of 575 bp was obtained for isolate KY1 and three bands of 531, 942 and 1245 bp for EM10 (Fig. 1C).



(A) Total DNA isolated from *Bacillus thuringiensis* isolates

(B) *cry*1 profile

(C) *cry*4 profile

FIGURE 1. Cry gene profile of native *Bacillus thuringiensis* isolates

DISCUSSION

The insecticidal activity of *B. thuringiensis* crystal protein was traditionally investigated by crude preparation of spore–crystal mixtures. In this study, crystal proteins of native bacterial isolates were compared with that of the reference strain HD1. The mortality response of larvae of *D. indica* showed high variability among the isolates.

TABLE 2. Details of amplicons obtained in *cry1* and *cry4* gene profile

Isolate	Details of amplicons					
	cry1			*cry4*		
	No. of bands observed	Mol. wt of amplicon (bp)	Expected size of amplicon (bp)	No. of bands observed	Mol.wt of amplicon (bp)	Expected size of amplicon (bp)
KY1	–	–	–	1	575	439
KY2	1	~250	277	–	–	–
KY3	1	~250	277	–	–	–
KY4	–	–	–	–	–	–
KY5	1	~250	277	–	–	–
KY6	1	~250	277	–	–	–
KK7	1	~250	277	–	–	–
KK8	1	~250	277	–	–	–
KK9	1	~250	277	–	–	–
EM10	–	–	–	3	531, 942, 1245	439
EM11	1	~250	277	–	–	–
HD1	1	~300	277	–	–	–
4Q1	–	–	–	1	524	439

Native isolates showed a lower mortality rate on test larvae as compared to reference strain HD1. All native isolates except EM11 recorded nearly 90% mortality in eight days. These could be considered as efficient isolates, according to Valicente and Barreto (2003) who reported that strains that killed more than 70% of larvae on 8th day were efficient ones. Some workers (Benhard *et al.*, 1997; Hossain *et al.*, 1997) have ranked bacterial isolates that caused more than 50% mortality when compared to standard strains as highly efficient and toxic (Chak *et al.*, 1994).

Statistical analysis of data carried out using Kendall's coefficient of concordance provides a measure of agreement or concordance between sample ranking or dependence of the samples (Siegal, 1975). Isolates ranked based on the mortality on 5th and 8th days confirmed that reference strain HD1 was more efficient in controlling larvae of *D. indica*. Among the native isolates, KK7, KK8, KK9 and KY2 were more efficient than the rest. Manimegalai *et al.* (2004) reported that susceptibility of lepidopteran larvae to parasporal crystal from various isolates of *B. thuringiensis* is one aspect of high selectivity of delta endotoxins towards insect species. Another reason for varying toxicity may be that certain insects have a low susceptibility for bacterial crystal proteins owing to the inefficient solubilization of crystals in the midgut. The delta-endotoxin is released by the bacterial isolate as protoxin and it should be converted to the toxic form, by the digestive enzymes in the midgut of insect larvae. Solubilization of crystal significantly enhances the toxic activity (Jaquet *et al.*, 1987). Since *B. thuringiensis* strains simultaneously produce more than one type of crystal proteins, bioassays can

be greatly influenced by the relative proportion of different proteins within the crystal. Hence it is difficult to accurately determine the spectrum of individual proteins causing toxicity.

Analysis of the potential of the strains against different orders of insect by bioassay is an exhaustive but time consuming process (Rajesh *et al.*, 2006). Of the alternative methods available, PCR analysis is considered to be good choice, as it allows rapid determination of the specific *cry* genes and has high sensitivity (Ceron *et al.*, 1995). The PCR amplification carried out with 11 isolates using *cry*1 gene specific primer indicated the presence of *cry*1 gene in the isolates KY2, KY3, KY5, KY6, KK7 KK8, KK9 and EM11 (Table 2). The *cry*1 gene is reported to be toxic to lepidopteran larvae. No amplification was obtained for isolates KY1, KY4 and EM10 indicating that they may contain cry gene other than *cry*1 gene group since they were also found toxic to *D. indica*. Isolates KY1 and EM10 and the reference strain 4Q1 showed amplification of cry4 gene alone indicating their potential against dipteran insects and presence of other gene not identified in the present investigation. Salem *et al.* (2006) identified *cry*1 and *cry*4 gene by producing fragments of 277 bp for *cry*1 and 439 bp for *cry*4 genes. Similar observations had been made by earlier workers (Carrozi *et al.*, 1991; Chak *et al.*, 1994). However, isolate KY4 remained negative for the amplification of *cry*1 and *cry*4 genes, and the mortality caused by the isolate can be attributed to the presence of gene other than *cry*1 and *cry*4 gene group which has to be investigated in future work. The genetic diversity and distribution of *cry* genes varied according to the regions from where they were collected. Thus the PCR analysis was not in full agreement with the results of the bioassay studies. Carrozi *et al.* (1991) suggested that novel isolates containing novel *cry* genes may give PCR products different in size relative to the standard or may completely lack PCR products. The report of 32 different *cry* gene groups by Crickmore *et al.* (1998) suggested that for identification of specific *cry* gene groups, the isolates should be examined using different sets of *cry* gene primers and also with primers specific for subgroups of each *cry* gene.

To conclude, the most obvious factors that contribute to the potency of delta-endotoxins are the origins of toxin (strain), the ability of gut juice to dissolve the protoxins and the intrinsic susceptibility of the insect to the toxin. The present study resulted in the identification of insecticidal activity of native isolates of *B. thuringiensis* active against *D. indica* and the presence of *cry*1 gene in eight of the 11 isolates studied. Two isolates had only *cry*4 gene and were still toxic to *D. indica* and one with neither *cry*1 or *cry*4 gene also killed the larvae. Emphasis must hence be laid on the identification and isolation of novel *cry* genes from bacterial strains which will be useful in pyramiding of different resistant genes.

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REFERENCES

Ben-Dov E. A., Zaritsky E., Dahan Z., Barak R., Sinai R., Manasherob A., Khamraev E., Troitskaya A., Dubitsky N. B. and Margalith (1997) Extended screening by PCR for seven *cry* group genes from field collected strains of *Bacillus thuringiensis*. Applied and Environmental Microbiology, 63: 4883–4890.

Benhard K., Jarret P., Meadows M., Butt J., Ellis D. J., Roberts G. M., Pauli S., Rodgers P. and Burges H. D. (1997) Natural isolates of *Bacillus thuringiensis*: Worldwide distribution, characterization and activity against insect pests. Journal of Invertebrate Pathology, 70: 59–68.

Carrozi N., Kramer V., Warren G., Evola S. and Koziel M. (1991) Prediction of insecticidal activity of *Bacillus thuringiensis* strains by polymerase chain reaction product profiles. Applied and Environmental Microbiology, 57(11): 3057–3061.

Ceron J., Ortiz A., Quintero R., Guereca L. and Bravo A. (1995) Specific PCR primers directed to identify *CryII* and *CryIII* genes within a *Bacillus thuringiensis* strain collection. American Society of Microbiology, 61: 3826–3832.

Chak K. F., Chao D. C., Tseng M. Y., Kao S. S., Tuan S. J. and Feng T. Y. (1994) Determination of *cry* type genes of *Bacillus thuringiensis* isolates from Taiwan. Applied and Environmental Microbiology, 60: 2415–2420.

Crickmore N., Zeigler D. R., Feitelson J., Schnepf E., Van Rie J., Lereclus D., Baum J. and Dean D. H. (1998) Revison of nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiology and Molecular Biology Reviews, 62: 807–813.

Dulmage H. T. (1970) Insecticidal activity of HD1, a new isolate of *Bacillus thuringiensis var. alesti*. Journal of Invertebrate Pathology, 15: 232–239.

Fietelson J. S. (1993) The *Bacillus thuringiensis* family tree. In: *Advanced engineered pesticides*, Kim L. and Marcel (Eds). Dekker Inc., NY, USA, 63–71.

Hossain M. A., Ahmed S. and Hoque S. (1997) Abundance and distribution of *Bacillus thuringiensis* in the agricultural soils of Bangladesh. Journal of Invertebrate Pathology, 70: 221–225.

Iriarte J., Dumanoir V. C., Bel Y., Porcar M., Ferrandis M. D., Lecadet M-M., Ferré J. and Caballero P. (2000) Characterization of Bacillus thuringiensis ser. Balearica (Serotype H48) and ser. Navarrensis (Serotype H50): two novel serovars in Spain. Current Microbiology, 40: 17–22.

Jaquet F., Hutter R. and Luthy P. (1987) Specificity of *Bacillus thuringiensis* delta endotoxin. Applied and Environmental Microbiology, 53: 500–504.

Kendall M. G. and Smith B. (1939) The problem of m Rankings. The Annual of Mathematical Statistics, 10(3): 275–287.

Kronstad J. W. and Whiteley H. R. (1986) Three classes of homologous *Bacillus thuringiensis* crystal-protein genes. Gene, 43: 29–40.

Lambert B., Hofte H., Annys K., Jansens S., Soetaert P. and Peferoen M. (1996) Novel *Bacillus thuringiensis* insecticidal protein with a silent activity against Coleopteran larvae. Bioscience, 42: 112–122.

Manimegalai S., Chandramohan N. and Jayarani S. (2004) Sex influenced Inheritance of *Bacillus thuringiensis cry*1Ac Resistance in American Bollworm *Helicoverpa armigera* (Hubner). Pesticide Research Journal, 16(4): 40–44.

Rajesh N. R., Alagawadi A. R. and Krishnaraj P. U. (2006) *Cry* diversity of *Bacillus thuringiensis* isolates of Western Ghat region and their bioefficacy against *Spodoptera litura* and *Helicoverpa armigera*. Entomon, 31(2): 89–98.

Salem H. H., Ali B. A., Tian H. H. and Qing D. X. (2006) Molecular characterization of novel *Bacillus thuringiensis* isolate with molluscicidal activity against the intermediate host of Schistosomes. Biotechnology, 5(4): 413–420.

Sambrook J. and Russel D. W. (2001) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, USA, 1541 pp.

Schesser J. H., Kramer K. J. and Bulla L. A. (1977) Bioassay for homogeneous parasporal crystal of *Bacillus thuringiensis* using the tobacco hornworm, Manduca sexta. Applied and Environmental Microbiology, 33: 878–880.

Siegal S. (1975) *Non Parametric Statistical Inference*, Marcel, Dekker, New York, 217 pp.

Travers S. R., Martin P. A. M. and Reichelderfer C. F. (1987) Selective process for efficient isolation of soil *Bacillus* spp. Applied and Environmental Microbiology, 53(6): 1263–1266.

Valicente F. H. and Barreto M. R. (2003) *Bacillus thuringiensis* survey in Brazil: Geographical distribution and insecticidal activity against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera:Noctuidae). Neotropical Entomolgy, 32(4): 639–644.

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Effect of fruit maturity on ovipositional behaviour of gravid female Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) infesting guava

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ABSTRACT: The ovipositional behaviour of Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) on immature, mature and ripe guava fruits was quantified. The parameters of behaviour observed were cleaning, body movement, searching, flight and oviposition. The temporal pattern of behavioural organization was examined by constructing an ethogram. More female fruit flies responded to ripe fruits than mature/immature fruits. Correlation analysis revealed significant association between successive behaviours (searching, oviposition, cleaning) which were observed more frequently on ripe and mature fruits than on immature ones indicating a lower acceptance of the latter.

KEYWORDS: *Bactrocera dorsalis*, ovipositional behaviour, guava, fruit maturity

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are important insect pests all over the world (White and Elson-Harris, 1992). Of the frugivorous tephritids, the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) [found mainly in the Oriental region] is a serious pest on a wide range of fruit crops in Taiwan, China and Indian subcontinent. It has also established in the Hawaiian Islands in 1945 (Pemberton, 1946) and in French Polynisia by 1996 (Vargas *et al.*, 2007). Given the tremendous economic importance of this species, studies have been biased to management. Progress has been made in managing the pest using methyl eugenol traps (Ishtiaq *et al.*, 1999), proteinaceous food baits (Cornelius *et al.*, 1999, 2000; Pinero *et al.*, 2009), insecticide applications (Anjum *et al.*, 2000) and integrated methods (Verghese *et al.*, 2004). Nevertheless, *B. dorsalis* continues to be a major concern causing huge pre and post harvest loss of fruits. To improve the current pre-harvest control strategies, it was felt that more research is needed in behavioural ecology and host selection process of gravid females. The objective of this

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study therefore was to test the influence of fruit maturity on the oviposition preference of gravid females.

MATERIALS AND METHODS

B. dorsalis culture was established in the laboratory from infested fruits collected from a guava (*Psidium guajava* L.) orchard at the Indian Institute of Horticultural Research (IIHR), Bangalore, south India. The fruit fly cultures were maintained on banana (*Musa* sp. cultivar *Elakki*) in a rearing room at 23 ± 1 °C (Jayanthi and Verghese, 2002) and adults eclosed were maintained age-wise in separate cages. From this culture, 20-day old gravid females were selected for the experiments.

Guava (cv. *Allahabad safeda*), a highly preferred host in the field and laboratory, was used as a test fruit. Fruits were collected from the orchards IIHR, washed and graded into three maturity groups based on rind color/hardness i.e., immature (hard-green), mature (semi hard-greenish yellow) and ripe (pale yellow with concomitant softening).

Oviposition behaviour of *B. dorsalis* was observed in wooden-framed cage with nylon mesh top, aluminum base and glass front. The test fruit was suspended halfway through the centre within the cage using a strong thread. Then, an individual gravid female fly was transferred from the mass culture and observed continuously for ten minutes. If the fly landed on the fruit in the first ten minutes of observation, then the succeeding behavioural responses were recorded continuously for next 30 min. The time taken for each activity was recorded using a digital clock. If the fly left the fruit/flew away after landing, within initial 10 min of observation then also the recordings were continued. In each maturity group, 600 gravid females were individually observed for their behavioural patterns. The fruits in which fruit flies exhibited ovipositor insertion were kept individually to confirm the presence of maggots.

To assess the stage of the guava fruit preferred by gravid females for oviposition, immature, mature and ripe fruits were exposed overnight to fruit flies. In no-choice tests, fruits of each maturity group ($n = 6$) were exposed to 20 pairs of fruit flies, separately. In choice tests, two fruits of each maturity group ($n = 2 \times 3{:}6$) were exposed to fruit flies (20 pairs) at a time. These were replicated eight times. After overnight exposure, the fruits from choice/no-choice tests were removed from the cage and each fruit was kept separately in plastic container containing finely sieved sand (5 cm thick) for pupation. Observations on number of pupae and adults per fruit were recorded.

Data on the sequence of oviposition behaviour recorded on 600 fruit flies for all the three maturity groups of guava fruits ($3 \times 600 = 1800$ flies) were compiled. An ethogram was constructed considering the behavioural components, frequency, flow, duration and pattern of oviposition behaviour of *B. dorsalis*, foraging for each of the maturity group, showing the transition probability for each behaviour considering oviposition as main behaviour. Here the transitional behaviours that preceded/succeeded the oviposition activity alone were included. The mean time spent

TABLE 1. Differential response of gravid females exposed to immature, mature and ripe fruits

Fruit maturity stage	Number of flies responded (out of 600)	χ^2/Tukey (q)	Presence of maggots after incubation
Immature	19	7.86*/3.99* (Immature–Mature)	Absent
Mature	40	8.49*/4.13* (Mature–Ripe)	Present
Ripe	69	30.65*/8.12* (Ripe–Immature)	Present
Overall comparison 2×3 contingency		31.81*	

* significant at $p = 0.05$

in each behaviour was calculated and a time budget prepared. Two-way ANOVA was carried out to determine interaction between fruit maturity and behavioural activities.

The data from choice and no-choice tests were also subjected to ANOVA to test differences in the mean pupal number and percent adult emergence for each maturity group. Least significant differences (LSD) were used for the comparison of means. A chi-square (2×3) and Tukey style multiple comparisons were carried out to analyze the differences in fruit fly response to different maturity groups (Jerrold, 1999). Correlation analyses between the times spent in each behaviour across the maturity groups (both pooled and individual) were carried out (Little and Hills, 1978).

RESULTS

Of the 600 flies released, data of 128 responding females were compiled.

Data presented in Table 1 show that fruit maturity significantly influenced host selection by gravid females for oviposition. The behavioural ethograms (Fig. 1) developed for all three maturity groups suggest that searching was the key behaviour which precedes or succeeds the oviposition activity in all the three groups. However, searching was more frequently observed in ripe fruits (396 times, in which 54.04% oviposition succeeded search and 45.96% oviposition preceded search), followed by mature fruits, where similar trend was observed (61 times, 60.66% oviposition succeeded search and 39.34% oviposition preceded search). The behaviour was least observed in immature fruits (13 times, where mostly search succeeded oviposition (53.85%) followed by search preceded oviposition (46.15%)).

The second predominant activity was cleaning of abdomen/ovipositor which followed the same order of frequency as in case of search, for all maturity groups: 19 for ripe, 12 for mature and 9 for immature. The data clearly showed that the main behaviours associated with oviposition were searching and cleaning (abdomen/ovipositor) and their frequencies were highest in the ripe fruits followed by mature and immature fruits. Overall, the ovipositional behaviour was dominated by a high frequency of search followed by cleaning (Fig. 1).

The mean proportion of time spent in each of the behavioural activity is summarized

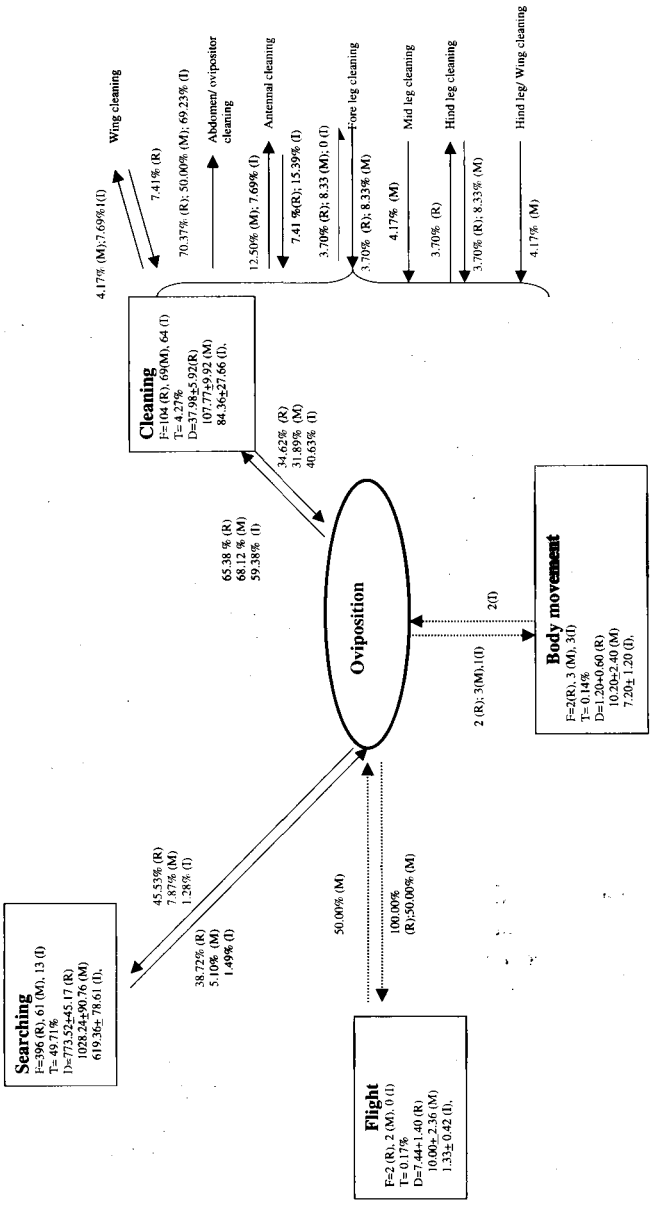

FIGURE 1. Ethogram of *B. dorsalis* showing the behavioural sequences preceding/succeeding the oviposition in ripe (R), mature (M) and immature (I) fruits. A solid line arrow represents a significant behavioural flow ($P < 0.05$), while a dotted line arrow represents an insignificant behavioural flow ($P > 0.05$). The figures adjacent to the arrows represent percentage of transitions from given behavioural events; T = percentage time devoted to each behavioural event; and D = mean (±SE) duration (seconds) of a single behavioural act (n = 128 i.e., 19, 40, 69 flies repectively on immature (I), mature (M), and ripe (R) fruits).

TABLE 2. Ovipositional preference of *B. dorsalis* for immature, mature and ripe fruits

Fruit maturity stage	Mean no. of pupae observed/fruit	Per cent adult emergence
Choice test		
Immature	0.00	0.00
Mature	15.38	70.33
Ripe	9.25	36.00
CD ($P = 0.05$)	4.51	5.91
No-choice test		
Immature	0.06	0.00
Mature	27.13	83.38
Ripe	19.15	41.61
CD ($P = 0.05$)	7.66	6.39

TABLE 3. Mean time spent by gravid female *B. dorsalis* in different behavioural activities (preceding/ succeeding oviposition) when exposed to different fruit maturity classes

Maturity class	Mean time spent in each behaviour (%)					Mean
	Cleaning	Movement	Searching	Flight	Oviposition	
Immature	4.37	0.09	35.80	0.02	57.83	98.11
Mature	5.83	0.28	54.04	0.17	36.93	97.25
Ripe	2.60	0.05	59.29	0.32	36.73	102.18
Pooled	3.87	0.13	54.16	0.23	39.92	

CD @ $P = 0.05$: Fruit stage, 9.78; Behaviour, 10.93; Interaction, 4.89

in Table 3 and the pooled means across different maturity groups showed that the gravid females spent relatively more time in searching (54.16%) which consisted of walking around/over the host fruit. In pooled analysis, oviposition was found to be the next major behaviour, which accounted for 39.92% of the total time and relatively small durations of time (3.87, 0.13 and 0.23%, respectively) were spent in behaviours like cleaning, body movement and flight. Similar trend was observed for the mature and ripe fruits. In case of the immature fruits, comparatively less time was spent in searching (35.80%) and more time in oviposition (57.83%); reverse trend was observed for mature and ripe fruits. Here, the flies spent more time in search (54.04% for mature and 59.29% for ripe fruits) and less time in oviposition (36.93% for mature and 36.73% for ripe fruits).

In immature fruits no maggots were found, whereas in mature and ripe fruits maggots were present (Table 1). This oviposition preference of gravid females was further supported by the results of choice/no-choice tests where the immature fruits were not preferred for oviposition by female fruit flies (Table 3). In choice tests, the matured fruits were preferred for oviposition, and more pupae were found in these

TABLE 4. Correlation analysis of observed behaviours (preceding/succeeding oviposition) exhibited by gravid female *B. dorsalis* on different fruit maturity classes

Immature				
Cleaning				
Movement	0.13			
Searching	0.09	−0.12		
Flight	−0.09	0.06	−0.08	
Oviposition	−0.27	0.05	−0.96**	−0.09
Mature				
Cleaning				
Movement	0.09			
Searching	0.04	0.30		
Flight	0.19	−0.11	0.37*	
Oviposition	−0.08	−0.29	−0.72**	−0.01
Ripe				
Cleaning				
Movement	0.30*			
Searching	0.29*	0.04		
Flight	0.17	−0.04	0.32**	
Oviposition	−0.36**	−0.11	−0.90**	−0.38**
Pooled				
Cleaning				
Movement	0.23*			
Searching	0.08	0.15		
Flight	0.07	−0.06	0.31**	
Oviposition	−0.19*	−0.19*	−0.84**	−0.27**

*, ** significant at $p = 0.05$ and 0.01, respectively

fruits. However, there was no significant difference in number of pupae in mature and ripe fruits. Similar trend was observed in no choice tests also. Nevertheless, the percent adult emergence was found to be higher in mature fruits than in ripe fruits in both choice and no-choice tests.

In pooled correlation analysis (Table 4), oviposition was significantly and negatively correlated with searching, flight, cleaning and body movement. Further, activities like cleaning and body movement; searching and flight were significantly and positively correlated. In individual correlation analyses, oviposition and search were significantly and negatively correlated for all the three fruit maturity groups. Significant correlations were not observed in the other behavioural activities in immature fruits. In the mature group, significant positive correlation was also observed between flight and searching.

In case of ripe fruits, correlation analysis showed significant negative relationship among oviposition and cleaning, oviposition and searching and oviposition and flight. Cleaning activity and movement, cleaning activity and searching, and searching and flight were positively correlated.

DISCUSSION

The role of fruit maturity in host acceptance for oviposition by gravid female was investigated in the present study. The gravid female displayed a relatively fixed pattern of behavioural sequence leading to oviposition (Fig. 1); however, frequency of behavioural transitions differed with fruit maturity groups. Gravid females exhibited high search rate in ripe fruits, followed by mature and immature fruits. Nevertheless, choice/no choice bioassays clearly showed that more pupae were observed in mature fruits compared to ripe fruits indicating higher oviposition in mature fruits (Table 2). The low adult emergence recorded in ripe fruits compared to mature fruits further supports the view that the ripe fruit does not last long enough to support the later larval stages (Table 2) causing forced pupation, often leading to low or premature adult emergence (Jayanthi and Verghese, 2002). Thus, females choose favourable host stage for oviposition, ensuring the growth and survival of the offsprings well into the adult stage. The importance of oviposition on favourable hosts is particularly important for tephritids where eggs are deposited beneath the fruit surface and larvae develop inside protective covering of the host fruit (Williamson, 1989).

Search and oviposition were found to be the predominant activities (Table 3) where females spent most of their time, irrespective of fruit stage. Females spent significantly more time in searching and oviposition on mature and ripe fruits compared to immature fruits. However, there was no significant difference for amount of time spent by females in searching and oviposition on mature and ripe fruits. In case of immature fruits, females spent significantly more time in oviposition than searching probably due to inadequate cues for oviposition. This was supported by the fact that no maggots were found in immature fruits exposed for oviposition.

Correlation analysis among the time spent in each activity by females showed more significant relationships in ripe fruits, followed by mature fruits, indicating that the duration of behaviour was influenced by fruit maturity. Consequently, when more time was spent in oviposition, lesser time was spent in cleaning, searching and flight, clearly indicating an efficient time budgeting. The initial attraction of gravid females to ripe fruits and high intensity of 'search' behaviour exhibited in these fruits as observed in the present study can be exploited as behavioural weak-links for their management. Elucidating the mechanism of higher attraction of gravid females to ripe fruits may lead to the development of some attractants useful in integrated management of the pest.

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REFERENCES

Anjum S., Razag M. and Yazadni M. S. (2000) Studies on seasonal activity and control of fruit flies (*Dacus* spp.) on mango (*Mangifera indica* L.) at Faisalabad, Pakistan. Arab Journal of Plant Protection, 18(2): 121–123.

Cornelius M. L., Duan J. J. and Messing R. H. (1999) Capture of oriental fruit flies (Diptera: Tephritidae) by protein-baited traps and fruit-mimicking visual traps in a guava orchard. Environmental Entomology, 28(6): 1140–1144.

Cornelius M. L., Nergel L., Duan J. J. and Messing R. H. (2000) Responses of female oriental fruit flies (Diptera: Tephritidae) to protein and host fruit odors in field cage and open field tests. Environmental Entomology, 29(1): 14–19.

Ishtiaq A., Farman U. and AlamKhan S. (1999) Efficacy of various insecticides and trap heights in methyl eugenol baited traps against fruit flies (*Bactrocera* spp.). Sarhad Journal of Agriculture, 15(6): 589–594.

Jayanthi P. D. K. and Verghese A. (2002) A simple and cost effective mass rearing technique for the tephritid fruit fly, *Bactrocera dorsalis* (Hendel). Current Science, 82(3): 266–268.

Jerrold Z. (1999) *In Biostatistical Analysis*, Fourth Edition, 564 pp.

Little T. M. and Hills F. J. (1978) *Agricultural Experimentation Design and Analysis*, Wiley, New York, 395 pp.

Pemberton C. E. (1946) A new fruit fly in Hawaii. Hawaiian Planter's Record, 50: 53–55.

Pinero J. C., Mau R. F. L. and Vargas R. I. (2009) Managing oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae), through spinosad-based protein bait sprays and sanitation in papaya orchards in Hawaii. Journal of Economic Entomology, In Press

Vargas R. I., Leblanc L., Rudolph P. and Eitam A. (2007) Impact of introduction of *Bactrocera dorsalis* (Diptera: Tephritidae) and classical biological control releases of *Fopius arisanus* (Hymenoptera: Braconidae) on economically important fruit flies in French Poynesia. Journal of Economic Entomology, 100(3): 670–679.

Verghese A., Tandon P. L. and Stonehouse J. M. (2004) Economic evaluation of the integrated management of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) in mango in India. Crop Protection, 23(1): 61–63.

White I. and Elson-Harris M. M. (1992) *Fruit flies of economic significance: their identification and bionomics*, CAB International in association with ACIAR, Canberra, 601 pp.

Williamson D. L. (1989) Biology and physiology; oogenesis and spermatogenesis. In: *Fruit Flies: their Biology, Natural Enemies and Control. World Crop Pests 3(A)*, Robinson A. S. and Hooper G. (Eds). Elsevier, Amsterdam, pp 141–151.

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Control of *Parthenium hysterophorus* L. using *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) in Ethiopia

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ABSTRACT: Laboratory experiments were conducted for fixing optimum number of beetles and larvae of the biocontrol agent, *Zygogramma bicolorata* Pallister required to control Parthenium weed and prevent its regeneration, under quarantine conditions at Ambo Plant Protection Research Center (APPRC), Ambo, Ethiopia during 2008-09. The beetles did not feed on any asteraceous test plant species under choice condition and they fed only on *Guizotia abyssinica* (34/254 leaves) under no-choice condition. No eggs were laid on two test plant species viz. *Bidens pachyloma* and *G. scabra*, and eggs laid on *B. ghedoensis* did not hatch. Eggs on remaining seven test plant species hatched but no larvae pupated, possibly due to antibiosis. Biology was thus not completed in any alternative hosts tested. Minimum percent adult mortality (3.33) was recorded on Parthenium whereas on remaining test plants it was higher (35 to 70). The number of 40 beetles per potted plant was found effective and optimum because it gave 99.33% defoliation in 20 days while a higher number of larvae (70 per pot) came on par with 40 beetles per pot. Defoliation of Parthenium by 40 adults per plant was judged as optimum because the defoliated plants did not regenerate. Although 100 beetles per plant could defoliate the plant faster a mean of 49 new shoots emerged per defoliated plant in 45 days.

KEYWORDS: host specificity, *Zygogramma bicolorata*, Parthenium, Asteraceae

INTRODUCTION

Parthenium, *Parthenium hysterophorus* L. is a herbaceous invasive weed belonging to Asteraceae family and is now widely distributed in east Africa, India, Australia and in many other countries. It was introduced into Ethiopia in 1970s and became a serious weed in arable and grazing lands (Tamado and Milberg, 2000). It is

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causing 40–97% reduction in sorghum grain yield in eastern Ethiopia (Tamado, 2001). This weed is also poisonous, pernicious, allergic and aggressively growing, posing serious threat to human beings and livestock. *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) is an effective bioagent to suppress this noxious weed. This beetle has a narrow host range within the Parthenium tribe Heliantheae and sub-tribe Ambrosiinae of Asteraceae family. *Z. bicolorata* damages the related plants of Parthenium such as *Verbesina encelioides* and *Xanthium occidentale* which belong to same tribe and sub-tribe. When beetle population increased in field and with the absence of Parthenium the beetle caused damage on sunflower but the larvae did not survive on it (Jayanth *et al.*, 1993). Julien and Graham (1997) reported that beetles caused heavy damage on normally rejected Parthenium plants also. Dhileepan *et al.* (2000) reported that *Z. bicolorata* reduced Parthenium seedling production from 73–90% in the field. This beetle was first imported officially from South Africa in 2007 to check the growth and spread of Parthenium and maintained under quarantine condition at APPRC, Ambo, Ethiopia. So far, no scientific evaluation of this weed killer has been carried out in Ethiopia. Hence, preliminary observations on safety and efficacy of *Z. bicolorata* and regeneration of defoliated Parthenium were carried out under quarantine condition in Ambo, Ethiopia.

MATERIALS AND METHODS

Host specificity test was conducted at Ambo Plant Protection Research Center (APPRC), Ambo, during 2008–09 under quarantine condition at 22 ± 3 °C and 65–70% RH. *Z. bicolorata* was reared on potted plants kept in cages made of wooden frames with polyethylene sheet on top and nylon mesh on four sides. Six economically important asteraceous plants viz. *Vernonia galamensis* (oil and varnish purpose), *Lactuca sativa* (salad crop), *Bidens pilosa* (medicinal as well as weed), *B. pachyloma* (ornamental in meskel and Ethiopian new year festival), *B. ghedoensis* (forage for animal as well as weed) and *Guizotia abyssinica* (major oil crop of Ethiopia), and four asteraceous weeds viz. *G. scabra*, *Flaveria trinervia*, *Tagetes minuta* and *Conyza bonariensis* were screened for their susceptibility to this beetle. The test plants and Parthenium were grown in plastic pots (20 cm × 18 cm) and each test plant was exposed to 20 adult beetles (10♂ and 10♀) in cages. Observation on defoliation percentage was recorded. Rearing cage with two holes and one hole were used for choice test and no-choice test, respectively. The treatment pots were arranged in completely randomized design with three replications.

Settling of insects on plants, feeding on the leaves, eggs laid and their hatching and subsequent development were recorded. Ten mated pairs of males and females (1:1) were released on caged plants and adults were removed after 10 days. For assessing the settling of beetles on plants and beetle mortality, 60 adults each were released per plant and number of beetles setting on the plant was counted and mortality recorded 10 days after release.

For assessing optimum number of adults and larvae to be used, Parthenium plants were grown in pots up to the height of 50 cm and enclosed in cages (1 m × 0.5 m ×

0.5 m) individually for confining the beetles. Eight treatments viz. 10, 20, 30, 40, 50, 60 and 70 adults and larvae per plant were used to determine the effectiveness in destroying the leaves. Total number of leaves of the plant per pot was counted before the release of insects and the number of leaves destroyed was recorded daily up to 45 days for adults and 15 days for larvae. Percentage of leaves consumed with reference to the pretreatment count was determined at the intervals of 5, 10, 15, 20 and 25 days after release of adults and 5, 10 and 15 days after release of larvae.

To study the regeneration of plants defoliated by the beetles, an experiment with three treatments was set up. In the first and second treatments, 40 and 100 beetles, respectively, were released per caged plant and the beetles were removed after complete defoliation. In the third treatment, the plant was manually defoliated. Number of shoots emerging was recorded at weekly interval and the last at 45 days, after complete defoliation. This experiment was set up in completely randomized design with six replications.

RESULTS AND DISCUSSION

Among the plants evaluated under no-choice condition, leaf damage by *Z. bicolorata* was observed only on *Guizotia abyssinica* (13% of leaves) and *Parthenium hysterophorous* (53%). Under choice condition, the beetles fed only on Parthenium (51%). Therefore, it can be expected that all the plants tested will be free from leaf damage under field situation. Slight defoliation by *Z. bicolorata* has already been reported on sunflower, *Helianthus annuus*, *Dahlia* spp. (Jayanth and Nagarkatti, 1987), *Xanthium occidentale* (McFadyen, 1992) and *X. strumarium* (Kumar, 1992). Non target feeding on sunflower was reported in India seven years after the introduction and establishment of *Z. bicolorata* (Chakravarthy *et al.*, 1996). On the basis of feeding behaviour (Jayanth *et al.*, 1998; Withers, 1999), life table (Viraktamath *et al.*, 2004) and field studies (Patel and Viraktamath, 2005), it appears that the chances of *Z. bicolorata* becoming a pest of *H. annuus* are negligible.

Data in Table 1 show that 85% of the released beetles settled on *P. hysterophorus* for egg laying, 60% on *G. abyssinica* and only 10 to 42% on the remaining plants. *G. pachyloma* and *G. scabra* fully deterred the egg laying. The highest number of eggs laid was on Parthenium (78), followed by *V. galamensis* (33) and *C. bonariensis* (28). On the remaining plants the number ranged from 8 to 18 only. The egg hatching percentage was highest on *V. galamensis* (78.79), followed by Parthenium (75.64), *G. abyssinica* (66.67) and *L. sativa* (61.11). The percentage hatching in remaining plants varied from 25 to 57.14 except in *B. ghedeonsis* in which no eggs hatched. The hatching larvae did not feed, grow and pupate on any test plant other than *P. hysterophorus*. The mortality of beetles exposed to the test plant was very low (3.33%) on Parthenium when observed 10 days after release while the percentages on all other plants were high (35 to 70%), apparently caused by starvation due to unsuitability of host. The adult neither fed nor laid eggs and larvae did not feed and survive on any plant except Parthenium and the ragweed, *Ambrosia* sp. (Asteraceae: Ambrosiinae) under choice condition (McFadyen and McClay, 1981) in Australia.

TABLE 1. Survival of *Zygogramma bicolorata* adults on asteraceous test plants under no-choice condition

Test plant	% of beetles settling on plant for oviposition	No. of eggs laid per plant	% of eggs hatched	% of adult mortality 10 d after release
Vernonia galamensis	25.00	33.00	78.79	60.00
Lactuca sativa	10.00	18.00	61.11	48.33
Bidens pilosa	10.00	14.00	57.14	61.67
B. pachyloma	20.00	0.00	0.00	70.00
Guizotia scabra	27.00	0.00	0.00	53.33
G. abyssinica	60.00	15.00	66.67	35.00
Flaveria trinervia	35.00	8.00	25.00	55.00
Tagetes minuta	25.00	8.00	37.50	51.67
Conyza bonariensis	20.00	28.00	50.00	60.00
Bidens ghedeonsis	42.00	12.00	0.00	40.00
Parthenium hysterophorus	85.00	78.00	75.64	3.33
CD ($P = 0.05$)	2.84	4.0	5.27	4.57

After five days of release of beetles, the maximum defoliation percentage (78.33) was recorded on plant with 70 beetles and it ranged from 0.85 to 72.33 in plants with 10 to 60 beetles (Table 2). After 10 days of release, the defoliation percentage was 95, 95.67, and 89.67, respectively, on plants with 70, 60, and 50 beetles and it ranged from 1.35 to 79.67 in plants with 10 to 40 beetles. More than 90% defoliation was recorded after 15, 20 and 25 days of release on plants with 40 to 70 beetles. The dose of 40 beetles per plant was found optimum because it gave significant defoliation (79.33 to 93.67%) after 10, 15 and 20 days of release. A further field study is needed on optimum dose considering weed biomass per unit area. Dhileepan *et al.* (2000) reported that Parthenium biomass was reduced when adults were maintained for long time under greenhouse and field condition. Jayachandra (1971) reported that 40 adults caused 90% Parthenium defoliation within 12–15 days.

When larvae were used instead of beetles, 70 larvae per plant caused 7% defoliation and 10 to 60 larvae caused 0.28 to 4% defoliation, after five days of release (Table 2). Similar trends of percent defoliation were recorded after 10 and 15 days. Highest defoliation was 11.2% and 14%, respectively, after 10 and 15 days of release, on 70 larvae per plant. Obviously the larvae were not effective in causing defoliation. The larval period was about 15 days and this short feeding period has also been noted as a constraint in larval effectiveness by McFadyen (1992).

Seven days after defoliation, plants which received 40 beetles per plant had an average of only one regenerated shoot/plant compared to 5.3 in those which received 100 beetles and 27.67 in hand defoliated plants (Table 3). The number of regenerated shoots was significantly greater in manually defoliated plants than on plants defoliated by beetles. Higher number of 100 beetles per plant defoliated the plant within short period (5–7 days) whereas the optimum number of 40 beetles defoliated the plant in a

TABLE 2. Defoliation of Parthenium by *Zygogramma bicolorata* at different densities of beetles and larvae per plant

No. of insects released/plant	Defoliation percentage at different intervals (days) after release				
	5	10	15	20	25
Beetles					
10	0.85	1.35	2.85	6.08	14.00
20	16.30	36.30	69.67	95.00	97.00
30	34.00	67.00	88.00	90.67	98.00
40	43.00	79.67	93.67	99.33	100.00
50	51.67	89.67	95.67	99.00	100.00
60	72.33	95.67	98.00	100.00	100.00
70	78.33	95.00	99.67	100.00	100.00
CD ($P = 0.05$)	6.90	7.23	5.70	1.90	0.90
Larvae					
10	0.28	0.60	1.10	—	—
20	0.51	1.06	2.50	—	—
30	0.60	2.10	3.90	—	—
40	1.40	2.80	5.90	—	—
50	2.87	4.60	6.40	—	—
60	4.00	5.80	9.00	—	—
70	7.00	11.20	14.00	—	—
CD ($P = 0.05$)	0.26	0.47	0.65	—	—

TABLE 3. Regeneration of Parthenium plants defoliated manually and by *Zygogramma bicolorata* adults

No. of beetles/plant	No. of shoots emerged after defoliation, at intervals (days)					
	7	14	21	28	35	45
100	5.3 (2.08)	15.67 (3.54)	23 (4.16)	31 (4.79)	48.67 (5.34)	49 (6.05)
40	1 (1.22)	1 (1.22)	1 (1.22)	1 (1.22)	1 (1.22)	1 (1.22)
Nil (Manual defoliation)	27.67 (5.27)	54.33 (7.32)	78 (8.84)	79 (8.81)	94.3 (9.67)	110 (10.47)
CD ($P = 0.05$)	1.67	2.49	2.88	2.55	4.32	4.11

Figures in parentheses are angular transformed values.

longer period (15–20 days). Regeneration of shoots was greater when defoliation was faster. Re-establishment of native vegetation of Parthenium after defoliation by this beetle has been reported by Jayanth and Visalakshy (1996) and Sridhar *et al.* (2005). Based on the conclusions from the present study, the government is on the way to release *Z. bicolorata* for controlling Parthenium in field condition.

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REFERENCES

Chakravarthy A. K., Cox M. L., Bhat N. S., Sridhar S and Thyagaraj N. E. (1996) Identification, host specificity and infestation of *Zygogramma conjuncta* Rogers on *Helianthus annus* L. In: *Biological and Cultural Control of Insect Pests: An Indian Scenario*, Ambrose D. P. (Ed). Adeline Publishers, Thirunelveli, Tamil Nadu, India, pp. 243–250.

Dhileepan K., Setter S. D. and McFadyen R. E. (2000) Response of the weed *Parthenium hysterophorus* L (Astreacae) to defoliation by the introduced biological control agent *Z. bicolorata*, (Coleoptera: Chrysomelidae. Biological Control, 19: 9–16.

Jayachandra M. (1971) Parthenium weed in Mysore state & its control. Current Science, 40: 568–569.

Jayanth K. P. and Nagarkatti S. (1987) Investigations on the host-specificity and damage potential of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) introduced into India for biological control of *Parthenium hysterophorus*. Entomon, 12: 141–145.

Jayanth K. P., Sukhadar M., Asokan R. and Visalakshy P. N. (1993) Parthenium pollen induced feeding by *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on sunflower (*Helianthus annuus*) (Compositae). Bulletin of Entomological Research, 83: 595–598.

Jayanth K. P. and Visalakshy P. N. G. (1996) Succession of vegetation after suppression of parthenium wwed by *Zygogramma bicolorata* in Banglore, India. Biological Agriculture and Horticulture, 12: 303–309.

Jayanth K. P., Visalakshy P. N. G., Chaudhary M. and Ghosh S. K. (1998) Age-related feeding by the parthenium beetle *Zygogramma bicolorata* on sunflower and its effecton survival and reproduction. Biocontrol Science and Technology, 8: 117–123.

Julien M. and Graham W. (1997) Biological control of weeds: Theory and practice application. Australian Centre for International Agricultural Research (ACIAR). Monograph, 49: 192.

Kumar A. R. V. (1992) Is the Mexican beetle *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) expanding its host range?. Current Science, 63: 729–730.

McFadyen R. E. (1992) Biological control against parthenium weed in Australia. Crop Protection, 24: 400–407.

McFadyen R. E. and McClay A. S. (1981) Two new insects for the biological control of parthenium weed in Queens land. In: *Proceedings of the 6th Australian weeds conference,1981 at Gold Coast*, Weed Science Society of Queensland.

Patel V. N. and Viraktamath C. A. (2005) Dispersal of parthenium biocontrol agent *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) in sunflower and *Parthenium hysterophorus* L. In: *Second International Conference on Parthenium Managemant*, Ramachandra Prasad T. V., Nanjappa H. V. and Devendra R. (Eds). University of Agricultural Sciences, Bangalore, India, 120–122.

Sridhar S., Basavaraja B. K. and Ganashaiah K. N. (2005) Temporal variation in relative dominance of *Parthenium hysterphorus* and its effect on native biodiversity. In: *Proceedings of the Second International Conference on Parthenium Management*, University of Agricultural Sciences, Bangalore, India.

Tamado T. and Milberg P. (2000) Weed flora in arable field of eastern Ethiopia with emphasis on the occurance of *Parthenium hysterophorus*. Weed Research, 40: 507–521.

Tamado T. (2001) *Biology and management of Parthenium (Parthenium hysterophorus L.) in Ethiopia*. PhD thesis. Swedish University of Agricultural Sciences, Uppsala.

Virakᵗamath C. A., Bhumannavar B. S. and Patel V. N. (2004) Biology and Ecology of *Zygogramma bicolorata* Pallister, 1953. In: *New Developments in the Biology of Chrysomelidae*, Joliver P., Santiago-Blay J. A. and Schmitt M. (Eds). SPB Academic Publishing, The Hague, The Netherland, pp. 767–777.

Withers T. M. (1999) Examining the hierarchy threshold model in a no-choice feeding assay. Entomologia Experimentalis et Applicata, 91: 89–95.

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Effect of *Bacillus thuringiensis israelensis* infection on the biochemical and hematological profile of the hemolymph of *Oryctes rhinoceros* [L.] (Coleoptera: Scarabaeidae) larva

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ABSTRACT: *Oryctes rhinoceros* [L.] (Coleoptera: Scarabaeidae) larvae exposed to cow dung experimentally contaminated with *Bacillus thuringiensis israelensis* (Bti) spores, at a density of 2 million spores/100 g, gave 100% mortality of the larvae within 72 h. During the course of infection, the larvae exhibited decreased mobility, food intake and body weight. Hemolymph of Bti infected larvae showed high viscosity, low volume, hyperproteinaemia and decrease in free amino acid content. Other hemolymph parameters such as uric acid and glucose content and activity of transaminases both AsAT and AlAT, showed an elevation but the activity of alkaline phosphatase showed a decrease. Total hemocyte count of the Bti infected larvae showed a sharp decline but the number of granulocytes showed a sharp increase. Various cytopathological changes such as distortion of cell shape, denucleation, abnormal staining and rupture of cell membranes were observed in plasmatocytes, following Bti infection.

KEYWORDS: *Bacillus thuringiensis israelensis*, *Oryctes rhinoceros*, hemocyte count, hyperproteinaemia

Oryctes rhinoceros [L.] (Coleoptera: Scarabaeidae) is the most potent pest of coconut palms. Several control measures have been devised since time immemorial but none of this could successfully eradicate this pest. The effective way of controlling this pest is to devise a method for controlling its larvae, which is the most vulnerable stage in its life cycle, which lives in decomposing organic matter. Experimental infection of 3rd instar larvae of *O. rhinoceros* with Bti has resulted in many hematological and biochemical changes in its hemolymph, suggesting the potential of Bti for control of

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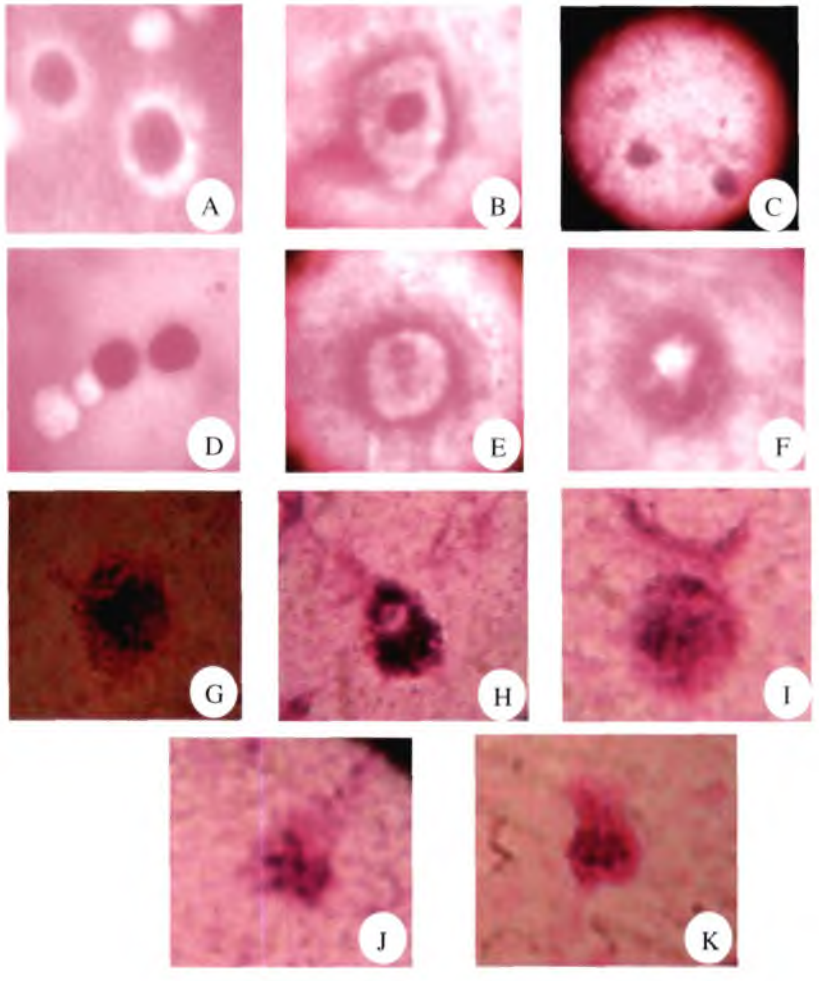

FIGURE 1. Normal hemocytes of *Oryctes rhinoceros* grub and cytopathological changes in Plasmatocytes following Bti infection. **A–F**, NORMAL HEMOCYTES: A, Prohemocytes; B, Plasmatocytes; C, Granulocytes; D, Adipohemocytes; E, Oenocytoids; F, Spherulocytes. **G–K**, CHANGES OBSERVED AMONG PLASMATOCYTES FOLLOWING BTI INFECTION: G, Abnormal staining; H, Distortion of cell shape, Cell enlargement and abnormal staining; I, Cell membrane rupturing; J-K, Cell membrane rupturing and denucleation.

the larvae and the present paper reports the results of this study. Similar changes have been reported in lepidopteran larvae after infection with various strains of *Bacillus thuringiensis* (Tripathi and Singh, 2000; El-Shershaby *et al.*, 2008).

The 3rd instar larvae of *O. rhinoceros* were maintained individually in plastic containers (11 cm × 8 cm) filled with 100 g cow dung. Actively feeding larvae after the 2nd week of moulting with an average weight of 9.4 g were used for the study. For

TABLE 1. Effect of Bti on the biochemical and hematological constituents of *Oryctes rhinoceros* larvae

Constituent	Spore concentration per 100 g cow dung			C.D.
	Nil (Control)	1 million	2 million	
Biochemical constituents of hemolymph				
Protein (μg/ml)	843.9 ± 21	961.81 ± 24	1252.32 ± 29	28.31
Aminoacids (μg/ml)	613.64 ± 32	517.05 ± 33	428.98 ± 3	19.52
Glucose (mg/100ml)	86.04 ± 3	93.30 ± 4	112.30 ± 6	3.12
Uric acid (mg/100 ml)	4.83 ± 0.2	7.34 ± 0.6	11.33 ± 0.8	0.71
Transaminases				
AsAT/GOT (micromoles OAA/min/mg protein)	12.02 ± 0.6	13.92 ± 0.8	14.26 ± 0.9	0.34
AlAT/GPT (micromoles pyruvate/min/mg protein)	9.21 ± 0.3	9.88 ± 0.6	11.02 ± 0.7	0.6
Alkaline phosphatase (K.A. units)	52.6 ± 1.3	43.20 ± 1.1	32.40 ± 0.98	1.6
Hemocyte count				
Total hemocyte count	10349 ± 80	6891 ± 61	3471 ± 72	70.35
Differential hemocyte count (%)				
Prohemocytes (PRs)	23.73 ± 0.23	17.29 ± 0.23	10.12 ± 0.18	0.21
Plasmatocytes (PLs)	16.46 ± 0.24	11.94 ± 0.21	7.21 ± 0.12	0.20
Adipohemocytes (ADs)	36.61 ± 0.14	27.32 ± 0.20	16.50 ± 0.17	0.18
Granulocytes (GRs)	13.68 ± 0.33	38.73 ± 0.20	64.62 ± 0.18	0.25
Spherulocytes (SPs)	3.17 ± 0.23	1.94 ± 0.64	0.64 ± 0.10	0.40
Oenocytoids (OEs)	6.35 ± 0.40	2.78 ± 0.58	0.91 ± 0.11	0.42

experimental infection of larvae, the desired concentration of Bti spores was mixed with 100 g cow dung and the larvae were allowed to feed on it. Hemolymph for biochemical and cytological studies was isolated by cutting the thoracic prolegs of the larvae at the 60th hour of infection. Standard procedures were used for biochemical estimations - protein (Lowry *et al.*, 1951), total free amino acid (TFAA) (Spies, 1957), glucose (Trinder, 1969), uric acid (Standard Assay Kit), transaminases (Reitman and Frankel, 1957) and alkaline phosphatase (Kind and King, 1954). THC and DHC were calculated following the method devised by Jones (1965). Data were analyzed statistically by ANOVA and critical difference (C.D.) was worked out (Daniel, 2006).

O. rhinoceros larvae on attack by Bti showed significant reduction in body weight and hemolymph volume together with increased viscosity of the hemolymph. The contents of protein, glucose and uric acid in the hemolymph showed a sharp elevation at the 60th hour of infection, while the gut of the infected larvae was empty. The results are shown in Table 1. Similar observations were reported by other investigators

in *Spodoptera litura* after infection with *Bacillus thuringiensis kurstaki* (Tripathi and Singh, 2002). The total free amino acid level (TFAA) in the hemolymph of a healthy larva was very high and this condition was considered to be an important character of the Class Insecta (Evans and Kaleysaraj, 1991, 1992). Contrary to this elevated level of TFAA, Bti infection caused a sharp decline of the same in the hemolymph of the larvae. A sudden decrease in the TFAA levels has been reported in *Spodoptera litura* and *Phthorimea operculella* larvae (Salama *et al.*, 1983, 1994) and in *Plodia interpuctella* (Aboul-Ela *et al.*, 1991) after infection with Bt. A sharp decline in TFAA together with the elevation of uric acid level up to 200–300% of its normal value was a clear indication of increased catabolism of TFAA following Bti infection. Hyperproteinaemia of the hemolymph was also a pathological change observed in *O. rhinoceros* larvae on severe infection by Bti. Hyperuricemia and hyperproteinaemia together with decrease in TFAA have pointed out another target in the host body, the DNA. Increased catabolism of nucleotides may also lead to hyperuricemia.

On severe infection by Bti, the activity of hemolymph transaminases showed a significant elevation, whereas the activity of alkaline phosphatase showed a steep decline. The elevation in the activity of transaminases in *Spodoptera littoralis* on infection with *B. thuringiensis kurstaki* has been reported by El-Shershaby *et al.* (2008). Elevated activity of transaminases was also observed in mammalian systems during various pathological conditions (Subramanium *et al.*, 1998). Decrease in the activity of alkaline phosphatase has been reported in cocoa pod borer when treated with Bti along with other botanical pesticides (*Vitex negundo*) (Santoso *et al.*, 2004).

The total hemocyte count (THC) of Bti infected larvae showed a significant decrease compared to control. Six different types of hemocytes, prohemocytes (PRs), plasmatocytes (PLs), adipohemocytes (ADs), granulocytes (GRs), oenocytoids (OEs) and spherulocytes (SPs) were observed in the hemolymph of the normal 3rd instar larvae of *O. rhinoceros*. Various cytopathological changes such as distortion of cell shape, abnormal staining pattern, cell enlargement, denucleation and rupturing of the cell membrane were observed mainly among the plasmatocytes of Bti infected larvae. Plasmatocytes and granulocytes are the phagocytic cells, where the former phagocytose non-self cells and the latter phagocytose dead cells (Eleftherianos *et al.*, 2009). The differential hemocyte count (DHC) revealed that the population of hemocytes showed numerical decrease but the granulocytes (GRs) showed a sharp elevation, up to 45%, during the course of infection by Bti. This indicated that granulocytes are playing a very important role in defense against pathogens through cell mediated and humoral mediated immunity, which requires a detailed study.

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REFERENCES

Aboul-Ela R., Kamel M. Y., Salama H. S., EL-Moursy A. and Abdel-Razek A. (1991) Changes in the biochemistry of hemolymph of *Plodia interpunctella* after treatment with *Bacillus thuringiensis*. Journal of Islamic Academy of Sciences, 4: 29–35.

Daniel W. W. (2006) In: *Biostatistics – A Foundation for Analysis in Health Sciences*, 7th edn, Georgia State University, Wiley and Sons (Asia) Pvt. Ltd., pp. 295–308.

Eleftherianos I., Xu M., Yadi H., Ffrench-Constant R. H. and Reynolds S. E. (2009) Plasmatocyte-spreading peptide (PSP) plays a central role in insect cellular immune defenses against bacterial infection. Journal of Experimental Biology, 212: 1840–1848.

El-Shershaby M., Farag N. A. and Ahmed A. I. (2008) Impact of *Bacillus thuringiensis* on protein content and enzyme activity of *Spodoptera litoralis*. Research Journal of Agriculture and Biological Sciences, 4: 861–865.

Evans D. A. and Kaleysa Raj (1991) Quassin, a mosquito larvicide with selective toxicity. Journal of Ecotoxicology and Environment Monitoring, 1: 243–249.

Evans D. A. and Kaleysa Raj (1992) Total protein, amino acid profile and certain related enzymes in adults and developing stages of *Culex quinquefasciatus* and effect of Quassin. Indian Journal of Comparative Animal Physiology, 10(1): 46–54.

Jones J. C. (1965) Hemocytes of *Rhodnius prolixus* (Stal). American Zoologist, 4: 282–294.

Kind P. R. N. and King E. J. (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. Journal of Clinical Pathology, 7: 322–326.

Lowry O. H., Roseborough N. T., Farr A. and Randall L. R. J. (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193(1): 265–275.

Reitman S. and Frankel S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28: 56–63.

Salama H. S., Sharaby A. and Ragaei M. (1983) Chemical changes in the hemolymph of *Spodoptera littoralis* (Lepidoptera: Noctuidae) as affected by *Bacillus thuringiensis*. BioControl, 28: 331–337.

Salama H. S., Ragaei M. and Sabbour M. (1994) Biochemistry of hemolymph of *Phthorimaea operculella* larva treated with *Bacillus thuringiensis*. Journal of Islamic Academy of Sciences, 7: 163–166.

Santoso D., Chaidamsari T., Wiryadiputra S. and de Maagd R. A. (2004) Activity of *Bacillus thuringiensis* toxins against cocoa pod borer, *Conopomorpha cramerella* larva. Pest Management Science, 60: 735–738.

Spies J. R. (1957) *Methods in Enzymology*, Academic Press, Inc Publishers, New York, pp. 468–471.

Subramanium A., Evans D. A., Rajasekharan S. and Pushpagadan P. (1998) Hepatoprotective activity of *Trichopus zeylandicus* extract against paracetamol induced hepatic damage in Rats. Indian Journal of Experimental Biology, 36: 385–389.

Trinder P. (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. Journal of Clinical Pathology, 22: 158–161.

Tripathi R. and Singh N. P. (2000) Effect of *Bacillus thuringiensis var. kurstaki* on biochemical changes in hemolymph of *Helicoverpa armigera* (Hubner) larva. Asian Journal of Experimental Sciences, 14: 31–34.

Tripathi R. and Singh N. P. (2002) Biochemical alterations in the hemolymph of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) infected Larva of *Spodoptera litura* (Fab). Asian Journal of Experimental Sciences, 16: 35–39.

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Breeding habitats and diversity of mosquitoes in Idukki District, Kerala State, India

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ABSTRACT: Mosquito-borne diseases, especially Dengue fever and Chikungunya have caused serious public health problems in Idukki district of Kerala State. The diversity of mosquito fauna, their distribution, breeding habitat and larval association were studied for the first time in the district. Immature stages and adult mosquitoes were collected from selected localities of higher and lower elevations of the district for a period of two years from February 2008. A total of 27 species, both vectors and non-vectors, belonging to nine genera viz, *Aedes, Anopheles, Armigeres, Coquillettidia, Culex, Heizmannia, Mansonia, Toxorhynchites* and *Uranotaenia* were recorded. Mosquito diversity and population varied with season and altitude. Maximum diversity was observed during monsoon season and higher number of mosquitoes at lower elevation. Larval habitats of 23 species of mosquitoes were detected during the study. Survey of larval habitats showed that the number of species supported by different habitat types varied from 2 to 13 and that the number of habitat selected by different species varied from 2 to 15.

KEYWORDS: breeding habitat, Chikungunya, mosquito diversity, Idukki

INTRODUCTION

Since 1970s several mosquito-borne diseases emerged or reappeared intensively in different parts of the world including India (Gubler, 1996, 1998). Similarly, over the past few years mosquito-borne diseases like Dengue fever (DF) and Chikungunya (CG) emerged in epidemic levels in Kerala, especially in central parts like Idukki district. DF first appeared in Idukki in 2001 (Kalra and Prasittisuk, 2004) and has now become almost endemic in the district. Idukki is one of the four districts with severe incidence of CG which was reported for the first time in Kerala during 2006–2007 (Kannan *et al.*, 2009). No literature is available on mosquitoes of the district. Awareness on mosquito diversity and their breeding habitat are essential for foreseeing the risk of various mosquito-borne diseases and adopting timely target specific control

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measures. The objectives of the present study were to investigate the diversity of mosquito fauna of Idukki district, their distribution, breeding habitats and larval associations of different species.

MATERIALS AND METHODS

Idukki district, located towards the centre of Kerala State, has a total area of about 5165 km^2 and lies between latitude 9°15′ and 10°21′ and longitude 76°37′ and 77°25′. Nearly 97% of the area is covered by mountains and more than 50% of the district is covered by forest, especially at the higher elevation. Low land (up to 20 m from MSL) is totally absent in the district. The annual rainfall varies from 2500 to 4250 mm.

Using stratified sampling, six localities each were selected from lower (up to 600 m from MSL) and higher elevations (above 600 m from MSL) of the district. Each selected locality was regularly sampled once in pre-monsoon (February–May), monsoon (June–September) and post-monsoon (October–January) season for a period of two years, from February 2008. Samples were also collected from same number of random localities. Thus 12 localities each were sampled from lower and higher elevations/season. In each locality six sites were visited.

Immature stages were collected from their natural breeding habitat using dippers in open sources and pipette in tree holes (Service, 1993). Small containers were emptied into plastic bag/sample bottle. Collections from each locality were maintained separately in suitable containers for adult emergence. Adult mosquitoes were collected from in and around human dwellings and animal sheds using net/aspirator and flash light between 1800 and 2200 hrs and 0700 and 1100 hrs (WHO, 1975). A total of four man hours was spent in each locality per observation. Adult specimens were identified using standard keys (Christopher, 1933; Barraud, 1934; Rao, 1974; Srivanakaran, 1976; Huang, 1977, 1979; Reuben *et al.*, 1994).

RESULTS AND DISCUSSION

Idukki district has a mosquito fauna of 27 species, belonging to nine genera viz, *Aedes, Anopheles, Armigeres, Coquillettidia, Culex, Heizmannia, Mansonia, Toxorhynchites* and *Uranotaenia* (Table 1). All the species were already reported from different parts of Kerala (Mariappan *et al.*, 1997; Hiriyan *et al.*, 2003; Arunachalam *et al.*, 2004; Thenmozhi *et al.*, 2007).

Genus *Culex* was predominant with a maximum of 12 species of which seven are known vectors in India. *Cx. vishnui, Cx. pseudovishnui* and *Cx. tritaeniorhynchus* are the common vectors of Japanese Encephalitis (JE). *Cx. whitmorei, Cx. bitaeniorhynchus* and *Cx. fuscanus* were also incriminated as vectors of JE. *Cx. tritaeniorhynchus* is identified as the primary JE vector in Kerala (Arunachalam *et al.*, 2004). *Cx. quinquefasciatus* is primary vector for bancroftian filariasis and suspected vector of JE (Mourya *et al.*, 1989). Non-vector mosquitoes of the study area include *Cx. pallidothorax, Cx. brevipalpis, Cx. minutissimus, Cx. univittatus* and *Cx. uniformis*.

TABLE 1. Mosquito fauna of Idukki district, Kerala, India

Species	Mean number of adult mosquitoes collected in each season									Total	%
	Pre-monsoon			Monsoon			Post-monsoon				
	Lower elevation	Higher elevation	Total	Lower elevation	Higher elevation	Total	Lower elevation	Higher elevation	Total		
Aedes (Aedimorphus) vexans	11.5		11.5	10.5		10.5	23.0	4.5	27.5	49.5	3.48
Aedes (Aedimorphus) vittatus[1]	7.0	2.5	9.5	18.5	9.5	28.0	10.0	2.5	12.5	50.0	3.52
Aedes (Finlaya) chrysolineatus	13.0	6.5	19.5	26.0	13.5	39.5	10.5	9.0	19.5	78.5	5.52
Aedes (Finlaya) niveus				10.0		10.0				10.0	0.70
*Aedes (Stegomyia) aegypti**[1,2]											0.00
Aedes (Stegomyia) albopictus[3,4]	88.5	50.5	139.0	69.0	29.0	98.0	40.5	22.0	62.5	299.5	21.10
Anopheles (Cellia) vagus	1.5	1.5	3.0				0.5		0.5	3.5	0.25
Armigeres (Armigeres) subalbatus	79.0	41.5	120.5	69.5	22.0	91.5	73.5	33.5	107.0	319.0	22.40
Coquillitidia (Coquillitidia) crassipes				2.5		2.5	2.0		2.0	4.5	0.32
Culex (Culex) bitaeniorhynchus[5]	24.0	7.0	31.0	7.0		7.0	11.5		11.5	49.5	3.48
Culex (Culex) pseudovishnui[5]	4.5		4.5	0.5		0.5	2.0		2.0	7.0	0.49
Culex (Culex) quinquefasciatus[6]	48.5	10.0	58.5	14.5	0.5	15.0	26.0		26.0	99.5	7.00
Culex (Culex) tritaeniorhynchus[7]	44.5	11.5	56.0	4.5		4.5	24.0		24.0	84.5	5.94
Culex (Culex) univittatus				1.5		1.5				1.5	0.11
Culex (Culex) vishnui	5.0		5.0	3.5		3.5	2.0		2.0	10.5	0.74
Culex (Culex) whitmorei[4]	3.5		3.5	0.5		0.5	1.5		1.5	5.5	0.39
Culex (Culiciomyia) pallidothorax	12.5	7.5	20.0	37.0	14.5	51.5	9.5	6.5	16.0	87.5	6.15

TABLE 1. Contd . . .

Species	Mean number of adult mosquitoes collected in each season									Total	%
	Pre-monsoon			Monsoon			Post-monsoon				
	Lower elevation	Higher elevation	Total	Lower elevation	Higher elevation	Total	Lower elevation	Higher elevation	Total		
Culex (Eumetanomyia) brevipalpis	4.5	3.5	8.0	27.0	8.0	35.0	9.5	5.5	15.0	58.0	4.08
Culex (Lophoceratomyia) minutissimus				1.0		1.0				1.0	0.07
Culex (Lophoceratomyia) uniformis	21.0	20.5	41.5	36.5	22.5	59.0	9.0	9.0	18.0	118.5	8.33
Culex (Lutzia) fuscanus	1.0		1.0	2.0		2.0	0.5		0.5	3.5	0.25
Heizmannia (Heizmannia) chandi				7.0	8.0	15.0				15.0	1.05
Mansonia (Mansonioides) annulifera[8]							1.0		1.0	1.0	0.07
Mansonia (Mansonioides) indiana[8]	1.5		1.5	6.0		6.0	1.5		1.5	9.0	0.63
Mansonia (Mansonioides) uniformis[6]	12.5		12.5	5.0		5.0	8.5		8.5	26.0	1.83
Toxorhynchites (Toxorhynchites) splendens				7.5	6.0	13.5	3.0	5.5	8.5	22.0	1.55
Uranotaenia (Pseudoficalbia) novobscura				4.5	1.5	6.0	0.5	1.5	2.0	8.0	0.56
TOTAL	383.5	162.5	546.0	371.5	135.0	506.5	270.0	99.5	369.5	1422.0	100

*obtained only as larvae; 1, primary vector of yellow fever; 2, primary vector of Dengue fever and Chikungunya; 3, secondary vector of Dengue fever and Chikungunya; 4, primary vector of Chikungunya and Dengue fever in Kerala; 5, secondary vector of Japanese encephalitis; 6, primary vector of filariasis; 7, primary vector of Japanese encephalitis; 8, secondary vector of filariasis

Of the six *Aedes* species recorded, *Ae. aegypti* is known as primary vector of DF and CG while *Ae. albopictus* is a secondary vector in different parts of world, including India (WHO, 1999; Jupp, 1988). However, in the State of Kerala, *Ae. albopictus* is recognized as the primary vector and plays a significant role in transmission of DF and CG (Kannan *et al*., 2009; Thenmozhi *et al*., 2007). *Ae. niveus* has been incriminated as secondary vector of DF in some parts of the world (Huang, 1979). *Ae. vittatus* and *Ae. aegypti* were identified as the main vectors of Yellow fever in many parts of the world (Bruce, 2005). *Ae. vexans* and *Ae. chrysolineatus* have no vector status. *Ae. aegypti* was obtained only as larva during monsoon season.

Genus *Mansonia* was represented by three species. *Ma. annulifera, Ma. indiana* and *Ma. uniformis* were incriminated as secondary vectors of JE in Kerala (Dhanda *et al*., 1997; Arunachalam *et al*., 2004). They have also been implicated as vectors of Brugian filariasis in the erstwhile Travancore area of Kerala as early as 1932 (Iyengar, 1938).

Genus *Anopheles, Armigeres, Coquillettidia, Heizmannia, Toxorhynchites* and *Uranotaenia* were represented by one species each (Table 1). *Ar. sabalbatus, Cq. crassipes, Hz. chandi, Tx. splendens* and *Ur. novobscura* are non-vectors as far as Kerala is concerned. Detection of *An. vagus* has great epidemiological significance, though it is not a vector for malaria. This observation indicates that *Anopheles* is still present in hilly tracts, which were once notorious for malaria in Kerala.

Distribution of mosquitoes varied with season and altitude (Table 1). Maximum species diversity was recorded during monsoon season (25 species), followed by post monsoon (22) and pre-monsoon seasons (18). Altogether 16 species were recorded from higher elevation and 27 species from lower elevation. Similar findings of fewer mosquito species at the higher elevation have already been reported (Rajput and Singh, 1988; Devi and Jauhari, 2004). The number of mosquitoes collected was also greater at lower elevation, accounting for about 72% of the total. Wide forest cover and low human as well as animal population at higher elevation may limit niche availability resulting in lower mosquito population. *Ae. niveus, Cx. univittatus* and *Hz. chandi* were found during monsoon season only.

Most of the species showed seasonal population fluctuation. *Ae. albopictus, Ar. sabalbatus, Cx. bitaeniorhynchus, Cx. pseudovishnui, Cx. tritaeniorhynchus, Cx. vishnui, Cx. whitmorei* and *Ma. uniformis* showed maximum population during pre-monsoon season, followed by a decline during monsoon. Population of *Cx. pallidothorax, Cx. brevipalpis, Cx. uniformis, Ae. chrysolineatus* and *Ae. vittatus* started building up during pre-monsoon and reached their maximum during monsoon season. But seasonal fluctuation of total mosquito population is not well pronounced, although higher numbers were collected from pre-monsoon season. *Ar. sabalbatus* and *Ae. albopictus* together (44%) played a major role in enhancing the total population during pre-monsoon. These two are the dominant species in the district and were abundant in all seasons at both higher and lower elevations. Together, they constituted about 43% of the total collection, followed by *Cx. uniformis* (8%), *Cx. quinquefasciatus* (7%), and *Cx. pallidothorax* (6%). *An. vagus, Ae. niveus,*

Cq. crassipes, *Cx. pseudovishnui*, *Cx. univittatus*, *Cx. vishnui*, *Cx. minutissimus*, *Cx. fuscanus*, *Ma. annulifera*, *Ma. indiana* and *Ur. novobscura* were the rare species.

Larval habitats of 23 species of mosquitoes were recorded during the study (Table 2). Larval stages of *An. vagus*, *Cq. crassipes*, *Cx. pseudovishnui* and *Ma. unnulifera* were not obtained during the study. Monitoring of larval habitats gives early estimates of future adult densities and provides the information necessary to eliminate mosquitoes at the source. Almiron and Brewer (1996) pointed out that type of habitat is the main characteristic that explains the observed variation among mosquito species. Many sampled species exhibited high degree of adaptive flexibility to various breeding habitats. *Ar. sabalbatus*, *Ae. albopictus* and *Cx. quinquefasciatus* had wider breeding habitat preference. *Ar. sabalbatus* surpassed all species by exhibiting adaptive flexibility to 15 habitats. The dengue vector *Ae. albopictus* is a container breeder and breeds in natural and artificial habitats. In the present study it was found to breed in 12 habitats. Breeding of *Ae. albopictus* in containers and latex collecting cups in rubber plantations of Kerala is well documented (Thenmozhi *et al.*, 2007; Sumodan, 2003). The present observation of breeding of *Ae. albopictus* in leaf axils of pineapple agrees with earlier reports (ICMR, 2006). Regu *et al.* (2008) reported arecanut leaf axils as a major breeding site of *Ae. albopictus* in areca plantations of Kerala. Breeding of *Ae. albopictus* in cow dung pits and leaf axils of plantain were not reported before. The notorious dengue vector *Ae. aegypti* was collected from coconut shells and latex collecting cups. Tree holes, leaf axils, coconut shells, coco pods, latex collecting cups etc provide abundant breeding habitat for mosquitoes, especially *Aedes*, during rainy season. *Culex* species were found to breed in a variety of habitats, as shown by Kumar and Vijayan (2005). *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* breed in small as well as large habitats. In conformity with the finding of Iyengar (1938), *Mansonia* was found to breed in habitat with floating vegetation such as pond/ground pool, rocky pools, canal and paddy fields. Rock pool, latex collecting containers and coconut shells gave shelter for more than 10 species while five types of habitats supported less than five species. During immature stages, many species co-existed in the same habitat. In the present study, 20 species showed association with respect to larval habitat (Table 3). *Ae. albopictus* and *Ar. sabalbatus* showed higher intensity of co-existence with eleven species each. Association varies with species and habitat (Table 3). The co-existence of more than one species in a habitat at a given time indicates that species share habitat requirements.

The present study is the first attempt to understand mosquito diversity and their breeding habitat in Idukki district of Kerala state. Forest cover, high rainfall, high relative humidity and moderate temperature along with crops such as rice, rubber, cocoa, pineapple, coconut and arecanut make suitable conditions for mosquito breeding and proliferation. The study revealed the presence of many vector mosquito species of various communicable diseases such as DF, CG, JE and filariasis. Source reduction through modification or eradication of habitat is essential for the control of mosquitoes and subsequently the mosquito borne-diseases.

TABLE 2. Breeding habitats of mosquito species in Idukki district, Kerala

Breeding habitat	*Ae. aegypti*	*Ae. albopictus*	*Ae. chrysolineatus*	*Ae. niveus*	*Ae.vexans*	*Ae. vittatus*	*Ar. sabalbatus*	*Cx. bitaeniorhynchus*	*Cx. brevipalpis*	*Cx. fuscanus*	*Cx. minutissimus*	*Cx. pallidothorax*	*Cx. quinquefasciatus*	*Cx. tritaeniorhynchus*	*Cx. univittatus*	*Cx. uniformis*	*Cx. visnui*	*Cx. whitmorei*	*Hz. chandi*	*Ma. uniformis*	*Ma. indiana*	*Tx. splendens*	*Ur. novobscura*	TOTAL
Canal/streams								+						+	+			+		+				5
Cess pit							+			+			+		+								+	5
Coconut shell	+	+	+	+		+	+		+		+	+	+	+		+							+	13
Cow dung pit		+			+		+																	3
Drains					+		+			+			+	+	+							+		7
Ground pool/pond							+	+		+			+	+			+			+	+		+	9
Latex collecting container	+	+	+	+		+	+		+		+	+	+	+		+							+	13
Leaf axils/fallen leaf sheath*		+		+																				2
Metallic containers		+	+				+				+	+	+											6
Mud pot		+	+			+	+		+					+		+			+			+		9
Paddy field																	+	+		+				3
Plant pot		+				+	+		+															4
Plastic container		+	+			+	+					+	+			+							+	8
Rocky pool						+	+	+				+	+	+		+		+		+	+			10
Tank		+				+	+	+	+			+	+	+				+						9
Tree hole/tree stump		+				+	+		+			+				+			+			+	+	9
Tyre		+	+				+						+											4
Others**		+		+			+					+	+											5
TOTAL	2	12	6	4	2	8	15	4	6	3	3	8	11	8	3	6	2	4	2	4	2	3	6	

Plus sign indicates presence. *pineapple, plantain, coconut, arecanut **cocoa pod, grinding stone, plastic sheets, glass, plates, etc.

TABLE 3. Association of mosquito species with respect to breeding habitat

Species	*Ae. albopictus*	*Ae. chrysolineatus*	*Ae. vittatus*	*Ae. vexans*	*Ar. sabalbatus*	*Cx. bitaeniorhynchus*	*Cx. brevipalpis*	*Cx. minutissimus*	*Cx. pallidothorax*	*Cx. quinquefasciatus*	*Cx. tritaeniorhynchus*	*Cx. univittatus*	*Cx. uniformis*	*Cx. vishnui*	*Cx. whitmorei*	*Hz. chandi*	*Ma. indiana*	*Ma. uniformis*	*Tx. splendens*	*Ur. novobscura*	TOTAL
Ae. albopictus		+	+		+		+	+	+	+			+			+			+	+	11
Ae. chrysolineatus	+		+		+																3
Ae. vittatus	+	+			+														+		4
Ae. vexans					+																1
Ar. sabalbatus	+	+	+	+		+		+	+	+	+	+							+		11
Cx. bitaeniorhynchus					+					+					+						3
Cx. brevipalpis	+								+				+								3
Cx. minutissimus	+				+								+								3
Cx. pallidothorax	+				+	+	+						+								5
Cx. quinquefasciatus	+				+	+					+			+				+			6
Cx. tritaeniorhynchus					+					+				+	+			+			5
Cx. univittatus					+																1
Cx. uniformis	+						+	+	+												4
Cx. vishnui										+	+										2
Cx. whitmorei						+					+										2
Hz. chandi	+																				1
Ma. indiana																		+			1
Ma. uniformis										+	+						+				3
Tx. splendens	+		+		+															+	4
Ur. novobscura	+																		+		2
TOTAL	11	3	4	1	11	4	3	3	4	6	5	1	4	2	2	1	1	3	4	2	

Plus sign indicates association.

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REFERENCES

Almiron W. R. and Brewer M. E. (1996) Classification of immature stage habitat of Culicidae (Diptera) collected in cordoba, Argentina. Memories do Instituto Oswaldo Cruz, Rio de Janeiro, 91: 1–9.

Arunachalam N., Samuel P. P., Hiriyan J., Thenmozhi V. and Gajana A. (2004) Japanese Encephalitis in Kerala, South India: Can *Mansonia* (Diptera: Culicidae) play a supplemental role in transmission. Journal of Medical Entomology, 41(3): 456–461.

Barraud P. J. (1934) *The Fauna of British India including Ceylon and Burma (Diptera; Culicidae) vol 5*, Taylor & Francis, London, pp. 463.

Bruce F. E. (2005) Mosquitoes, the Culicidae. In: *Biology of Disease Vectors*, William C. M., Jerome E. F., Henry H. H., Janet H., Stephen H., Anthony A. J., Boris K. and Chester G. M. (Eds). Academic press, Elsevier, pp. 95–111.

Christopher S. R. (1933) *The Fauna of British India including Ceylon and Burma (Diptera; Culicidae), vol 4*, Taylor & Francis, London, pp. 371.

Devi N. P. and Jauhari R. K. (2004) Altitudinal distribution of mosquitoes in mountainous area of Garhwal region: Part I. Journal of Vector Borne Diseases, 41: 17–26.

Dhanda V., Thenmozhi V., Kumar N. P., Hiriyan J., Arunachalam N., Balasubramanian A., Elango A. and Gajanana A. (1997) Virus isolation from wild caught mosquitoes during Japanese Encephalitis outbreak in Kerala in 1996. Indian Journal of Medical Research, 106: 4–6.

Gubler D. J. (1996) The global resurgence of arboviral diseases. Transactions of the Royal Society of the Tropical Medicine and Hygiene, 90: 449–451.

Gubler D. J. (1998) Resurgent vector-borne diseases as a global health problem. Emerging Infectious Diseases, 4(3): 442–450.

Hiriyan J., Arunachalam N., Philip Samuel P., Thenmozhi V., Gajanana A. and Satyanarayana K. (2003) Studies on the mosquito fauna in the Japanese Encephalitis prone area in Kerala, India. Entomon, 28(2): 139–146.

Huang Y. M. (1977) Medical Entomology Studies VIII. Notes on the taxonomic status of Aedes vittatus (Diptera: Culicidae), *Contributions of the American Entomological Institute*, pp. 132.

Huang Y. M. (1979) Medical Entomology Studies XI. The Subgenus Stegomyia of Aedes in Oriental region with key to species (Diptera: Culicidae), *Contributions of American Entomological Institute*, pp. 79.

ICMR (2006) Dengue in Kerala: a critical review. ICMR bulletin, 36(4-5): 14–22.

Iyengar M. O. T. (1938) Studies on the Epidemiology of Filariasis in Travancore. Indian Medical Research Memoirs, 30: 150–168.

Jupp P. G. and McIntosh B. M. (1988) Chikungunya virus disease. In: *The Arboviruses: Epidemiology and Ecology*, Monath T. P. (Ed). Boca CRC, Raton, FL, USA, vol 2: pp. 137–157.

Kalra N. L. and Prasittisuk C. (2004) Sporadic prevalence of DF/DHF in the Nilgiri and Cardamom Hills of Western Ghats in South India: Is it a seeding from sylvatic Dengue Cycle-A Hypothesis. Dengue Bulletin, 28: 44–50.

Kannan M., Rajendran R., Sunish I. P., Balasubramaniam Arunachalam N. and Paramasivan R. (2009) A study of Chikungunya outbreak during 2007 in Kerala, South India. Indian Journal of Medical Research, 129: 311–315.

Kumar Y. S. and Vijayan V. A. (2005) Mosquito fauna and breeding habitats in the rural areas of Mysore and Mandya districts, Karnataka state, India. Entomon, 30(2): 123–129.

Mariappan T., Arunachalam N., Somachary N. and Reddy C. M. R. (1997) A note on the mosquito fauna of Kochi and its adjoining islands in Kerala. Entomon, 22(2): 141–145.

Mourya D. T., Ilkal M. A., Mishra A. C., Jacob P. G., Pant U. and Ramanujam S. (1989) Isolation of Japanese Encephalitis virus from mosquitoes collected from Karnataka state India from 1985–1987. Transactions of the Royal Society of Tropical Medicine and Hygiene, 83: 550–552.

Ragu K., Rajendran R., Tamilselvan M. and Ganesh C. T. (2008) Shed leaf sheath of areca nut palm as a major breeding source of *Ae.albopictus* Skuse(Diptera) in Kerala. Hexapoda, 15: 111–113.

Rajput K. B. and Singh T. K. (1988) Vertical distribution of mosquitoes in Manipur. Entomon, 13 (3&4): 295–301.

Rao T. R. (1974) *The Anopheles of India*, Malaria Research Centre, New Delhi, pp. 505.

Reuben R., Tewari S. C., Hiriyan J. and Akiyama J. (1994) Illustrated key to species of Culex (Culex) associated with Japanese encephalitis in southeast Asia (Diptera: Culicidae). Mosquito Systematics, 26(2): 75–96.

Service M .W. (1993) *Mosquito Ecology: Field Sampling Methods*, 2nd edn, Applied Science, Elsevier, UK, pp. 998.

Srivanakaran S. In: *Medical Entomology Studies—IIIA.(1976) Revision of the subgenus Culex in the Oriental region (Diptera: Culicidae)*, Contributions of the American Entomological Institute, vol. 12: pp. 272.

Sumodan P. K. (2003) Potential of rubber plantation as breeding source for *Aedes albopictus* in Kerala. Dengue Bulletin, 27: 197–198.

Thenmozhi V., Hiriyan J. G., Tewari S. C., Samuel P. P., Paramasivan R. and Rajendran R. (2007) Natural vertical transmission of dengue virus in *Aedes albopictus* (Diptera: culicidae) in Kerala, a southern Indian state. Japanese Journal of Infectious Diseases, 60: 245–249.

WHO (1999) *Regional Guideline on Dengue and Dengue Hemorrhagic Fever Prevention and Control*, World Health Organization SEARO Publication, Delhi.

WHO (1975) *Manual on practical entomology in malaria vector bionomics and organization of antimalaria activities*. Pt I&II. Offset Publication, Geneva, pp. 160 (pt I) & pp. 191 (pt II).

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Description of a new species, *Memantis yercaudensis* (Mantodea: Amelinae) from Tamil Nadu, India

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ABSTRACT: *Memantis yercaudensis* sp. nov. from Yercaud, Tamil Nadu, India is described and illustrated. Modified key to the Indian species of the genus *Memantis* Giglio-Tos 1915 is also provided. *M. yercaudensis* sp. nov. is related to the species *M. gardeneri* Werner 1931 by having the same number of external spines on fore tibia and prominent lateral lobe of vertex but differs from it by the presence of 10/11 internal spines on the fore tibia instead of 9 and bifurcation of the first branch of cubitus of hindwing.

KEYWORDS: *Memantis yercaudensis*, new species, Yercaud, India, Mantodea, Amelinae

INTRODUCTION

The genus *Memantis* Giglio-Tos (1915) comes under the subfamily Amelinae which includes small or medium sized mantids, ranging between 11 mm and 30 mm. This genus was erected by Giglio-Tos (1915) on the basis of a type specimen, *Mantis fuliginosa* Thunberg (1815). The distribution of this genus is limited to India, Myanmar and Nepal. The recent book by Ehrmann (2002) indicates that four species, *Memantis anomala* Lombardo (1993), *M. fuliginosa* Thunberg, (1815), *M. gardeneri* Werner (1931), and *M. minor* Werner (1931), belong to this genus. Of these, *M. anomala* was reported from Nepal by Lombardo (1993) but other three species were reported from India. *Memantis yercaudensis* sp. nov is described and illustrated here with a modified taxonomic key to the genus *Memantis* Giglio-Tos (1915).

MATERIALS AND METHODS

The detailed study of all specimens collected was carried out using Leica EZ4D Stereo Zoom Microscope and images were taken using inbuilt digital camera of the

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microscope. Based on the observations, a description has been prepared; the format of the description follows Mukherjee *et al.* (1995); drawings were made using Camera Lucida mounted on LeicaMZ6 Stereo Zoom Microscope. Specimens are deposited in Entomology Research Laboratory, Providence Women's College, Calicut, Kerala, India and eventually will be transferred to Zoological Survey of India, Calicut Station.

RESULTS

Description

Genus: *Memantis* Giglio-Tos, 1915

Memantis Giglio-Tos, 1915. Bullettino della Societa Entomologica Italiana, 46: 163. Type-species: *Memantis fuliginosa* (Thunberg, 1815).

Diagnostic characters: Frontal sclerite transverse, smooth; superior border arched or truncated at middle. Pronotum shorter than fore coxa; lateral corners without tooth; Metazona a little longer than prozona, constricted posteriorly, with two rounded tubercles on mid-posterior borders; bosses of pronotum much pronounced. In fore legs, femora almost triangular, superior edge arched, with 4 external spines and with fine spinules; proximal two external spines closer to each other; 4 discoidal spines; fore tibia with 9–11 external and 9–10 internal spines. Hind metatarsus almost same in length or a little shorter than all other tarsal segments together. Fore wing in male longer and in female shorter than abdomen. (Diagnosis of genus by (Mukherjee *et al.*, 1995)).

Key to the Indian species of genus *Memantis* Giglio-Tos

1. Discoidal area of hind wing blackish in male and reddish in female .... *M. fuliginosa* (Thunberg)
– Hind wing hyaline 2
2. Lateral lobes of vertex prominent. Ist branch of cubitus of hind wing 2-3-branched 3
– Lateral lobes of vertex not prominent. Ist branch of cubitus of hind wing 4-branched ..... *M. minor* Werner
3. Ist branch of cubitus of hind wing 2-branched, foretibia with 10/11 internal spines ..... *Memantis yercaudensis* sp. nov
– Ist branch of cubitus of hind wing 3-branched, foretibia with 9 internal spines .. .. *M. gardeneri* Werner

***Memantis yercaudensis* sp. nov** (Fig. 1, Table 1)

Holotype: Male (Fig. 1(i)), Body length 18.5 mm.

Colour: Body generally testaceous. Eyes fuscous, vertex with dark brown patches, ocelli dark brown at base; ventral side of the fore leg with brown dots. A dorsomedian

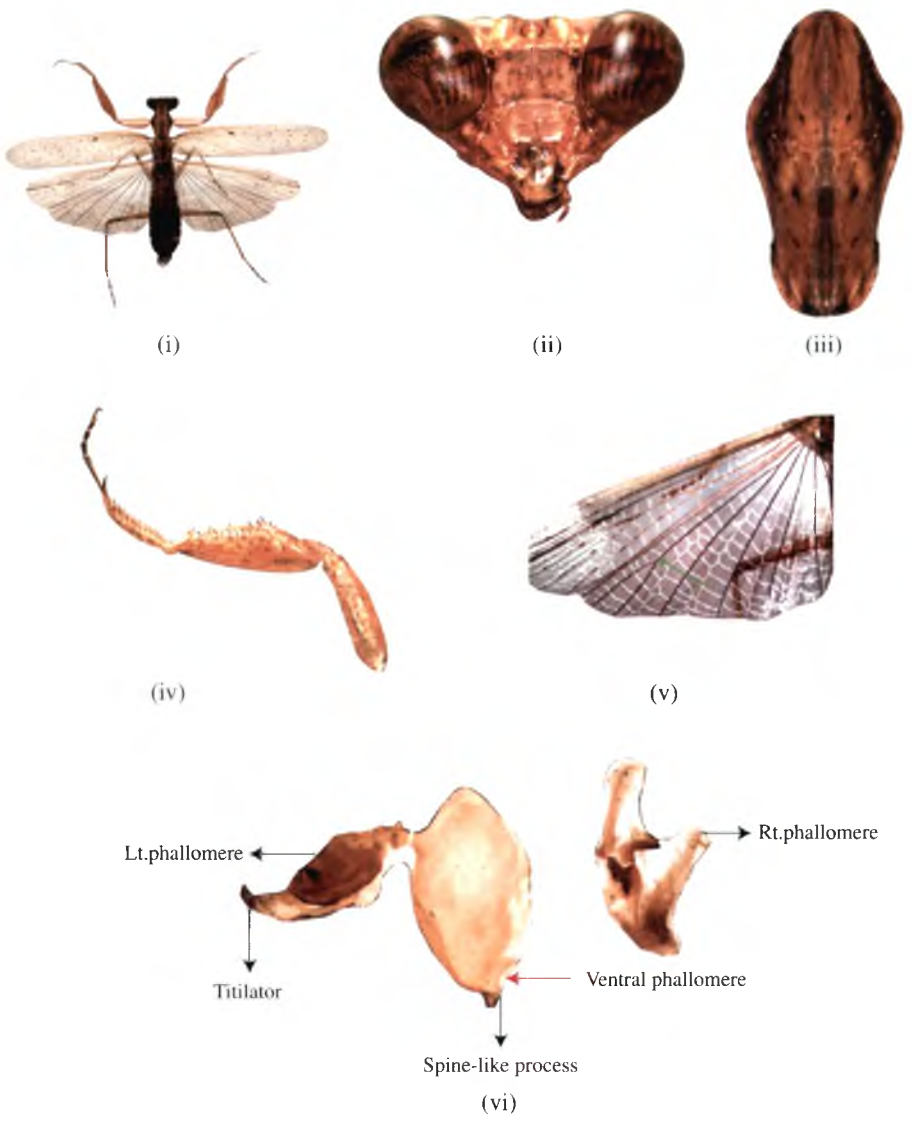

FIGURE 1. *Memantis yercaudensis* sp.nov. (i) Entire view; (ii) . Head: frontal view; (iii). Pronotum; (iv). Fore leg; (v). Hind wing showing cubitus vein; (vi). Male genitalia.

dark brown strip present on pronotum, lateral margin also with dark brown dots; forewing with fuscous dots on major veins, stigma dark brown; hind wing smoky, a blackish streak present on distal part of radius vein, anal veinlets whitish; abdomen dark brown at distal half.

Head (Fig. 1(ii)): Vertex smooth, lateral lobe prominent; ocelli small, frontal sclerite more than 1.5× wider than high, upper margin arched; antennae sparsely setaceous, longer than body; eyes round.

Pronotum (Fig. 1(iii)): Small sized, slightly shorter than forecoxa, supracoxal dialation round, prozona finely dentate at lateral side, metazona a little longer than prozona with a faint dorsomedian carina with two flat bosses posteriorly; metasternum with a median longitudinal groove.

Fore leg (Fig. 1(iv)): Coxa a little swollen ventrally, apical lobe divergent, both anterior and posterior margins sparsely setaceous; lower side of trochanter setaceous; femur a little longer than coxa, with 13 internal, 4 discoidal and 4 external spines: proximal two external spines a little closer; claw groove proximally placed; tibia short with 10/11 internal, 10 external spines.

Mid and hind leg: Both mid and hind femora with an apical spine; hind metatarsus almost as long as all other tarsal segments together.

Wings: Both wings longer than abdomen. Fore wing semi-transparent, costal area broad at proximal half, narrowed distally; radial vein trifurcates at discoidal area; median vein bifurcates at discoidal area. Hind wings hyaline with white transverse veinlets at anal area; first branch of cubitus bifurcated (Fig. 1(v)).

Abdomen: A little broad, supra anal plate triangular; cerci setaceous.

Genitalia: With three phallomeres or lobes, right, left and ventral (Fig. 1(vi)). The right phallomere is dorsally placed, triangular, bilobed and flat with two prongs and a hook-like process laterobasally. The left phallomere, placed dorsally, has an outwardly curved titilator, a peudophallus and basal sclerite. The ventrally placed ventral phallomere is flat and plate-like with a single small spine like process at the distal tip, unlike in other mantids, especially *M. anomala*, in which the ventral phallomere has two branches at distal end, one pointed posteriorly and the other anteriorly (Lombardo, 1993).

Female: Unknown.

Remarks: The two paratypes studied have no remarkable variation from the holotype. Only slight differences in measurements.

Specimens examined: The specimens were collected from Yercaud, Shevaroy Hills, Tamil Nadu, India [11.7794° N and 78.2034°E].

Holotype ♂. India: Tamil Nadu; Shevaroy Hills, Yercaud, 19-v-2009; Altitude: 1370m, Vyjayandi & party. Other specimens examined: 2 ♂. India: Tamil Nadu; Yercaud, 19-v -2009; Vyjayandi & party.

Habitat: Bushy vegetation of herbs and shrubs.

TABLE 1. Measurements of *Memantis yercaudensis* sp. nov. (in mm)

Specimen	Total length	Pronotum	Fore leg			Middle leg			Hind leg			Fore wing	Hind wing
			Coxa	Femur	Tibia	Coxa	Femur	Tibia	Coxa	Femur	Tibia		
Holotype	18.5	4	4	4.5	3	2	5	4	3	4	4.5	19	16
Paratype I	18	4	3.5	5	2.5	2	4	3	2	5	5	18	17
Paratype II	17	4	3.5	4.5	3	2	4	3	2	5	4	17.5	15

DISCUSSION

In *M. gardeneri*, 1st branch of cubitus of hind wing is 3-branched but in the new species 1st branch of cubitus of hind wing is 2-branched. *M. gardeneri* has fore tibia with 9 internal spines. In *M. yercaudensis* sp. nov. fore tibia has 10/11 internal spines. In *M. gardeneri*, disc of pronotum has 4 pairs of bosses of which 2nd and 4th are very prominent but in *M. yercaudensis* sp. nov. disc of pronotum has two bosses only at mid dorsal posterior border. Fore femora of *M. gardeneri* possess a row of tubercles externally but this is absent in *M. yercaudensis* sp. nov.

Memantis yercaudensis sp. nov. differs from *M. anomala* in having smooth vertex without any characteristic tubercles at occiput, straight anterior margin of fore femur, and ventral phallomere of the copulatory organ (Fig. 1(vi)) with small spinous process at the distal end. In *M. anomala* the ventral phallomere has two branches at distal end (Lombardo, 1993). Examples of *M. gardeneri* located at Z.S.I., Kolkata were not available for dissection of genitalia.

Etymology

The species is named after the place of collection, Yercaud.

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REFERENCES

Ehrmann R. (2002) Gottesanbeterinnen der Welt. In: *Natur und Tier- Verlag GmbH Münster*, Berlin, pp. 219.

Lombardo F. (1993) Studies on the Mantodea of Nepal (Insecta). Spixiana, 16(3): 204–205.

Giglio-Tos E. (1927) *Das Tierreich. Orthoptera Mantoidea*, Walter de Gruyter & Co., Berlin, vol 50, pp. 707.

Mukherjee T. K., Hazra A. K. and Ghosh A. K. (1995) The mantid fauna of India (Insecta: Mantodea). Oriental Insects, 29: 274–276.

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Description of seven new species of *Liriomyza* Mik (Diptera: Agromyzidae) from India

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ABSTRACT: Seven new species belonging to the genus *Liriomyza* Mik (Diptera: Agromyzidae) from India (4 from Kerala (Western Ghats), 2 from West Bengal (Himalayas) and 1 from Madhya Pradesh) were recorded. Key to species of the genus *Liriomyza* Mik from India, reprensented by 19 species of which seven are new to science is also given.

KEYWORDS: Agromyzidae, Diptera, India, *Liriomyza*, new species

INTRODUCTION

The genus *Liriomyza* Mik (Diptera: Agromyzidae) has 12 recognized species from India; with the addition of seven new species there will be a total of 19 species. The seven species described below are new to science. Garg (1971) from India described five species of genus *Liriomyza* Mik. Singh and Ipe (1973) described four species and reported three known species from India which were *Liriomyza brassicae* (Riley) Frick, *L. compositella* (Malloch) Spencer and *L. sonchi* Hendel. They also gave the key to all the species reported from India. All of these species were collected mostly from the high altitudes of Himalayan region, Western Ghats of India and from the Gangetic Plains. In the present study, seven new species are described which are also collected from the same regions and geographical conditions.

MATERIALS AND METHODS

The larvae, pupae and adult flies were collected from infested plants. The larvae were preserved in Pample's fluid and finally mounted in Canada balsam. Pupae were kept

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in glass tubes with their openings covered by thin muslin. The soil from under the plant was collected and placed at the bottom of the tubes. It proved to be useful in case of *Liriomyza*, which pupates in soil. After separation and dehydration male genitalia were mounted finally in the Canada balsam. For the wings the slides were prepared by dipping the wings into xylol and thereafter mounted in Canada balsam. The host plants were collected and pressed in the field and transferred to the herbarium sheets in the laboratory for identification. All the diagrams were made with the help of Camera lucida and graph eye piece.

ABBREVIATIONS

acr, acrosticals; *dc*, dorsocentral bristle; *h*, humeral bristle; *inv*, inner vertical bristle; M_{1+2}, median vein 1 + 2; M_{3+4}, median vein 3 + 4; *m-m*, medial cross vein; *npl*, noto plurals; *ori*, lower orbital bristle; *ors*, upper orbital bristle; *ovt*, outer vertical bristle; *pa*, postalar bristle; *prs*, presutular bristle; *pvt*, post vertical bristle; R_1, radius vein 1; R_{2+3}, radius vein 2 + 3; R_{4+5}, radius vein 4 + 5; *r-m*, radio-medial cross vein; *sc*, scutellar bristle.

RESULTS AND DISCUSSION

Key to species of the genus *Liriomyza* Mik from India

(Modified from Singh and Ipe (1973))

1. *r-m* distad of mid point of discal cell 2
   - *r-m* before or at mid point of discal cell 5
2. Frons projecting above or infront of eye in profile 9
   - Frons not projecting or at level with eye in profile 3
3. Lunule, sunken, semicircular; *acr* in four rows; ultimate sector of M_{1+2} three times the penultimate ..... ***brassicae* (Riley)**
   - Lunule compicously higher than semicircular or acutely triangular 4
4. *acr* in 8 rows of 4 abreast; ultimate and penultimate sectors of costa in the ratio 1:0.8 ..... ***alticola* Singh and Ipe**
   - *acr* in 5 rows of 4 abreast; ultimate and penultimate sectors of costa equal ..... ***kalimpongensis* sp. nov**
5. *r-m* before mid point of discal cell 7
   - *r-m* at mid point of discal cell 6
6. Post gena extending entire length of eye behind; costal segments ultimate and penultimate equal; *acr* in 6 rows of 4 abreast; lunule semicircular ..... ***apsora* Singh and Ipe.**
   - Post gena not extending entire length behind eye; lunule distinctly higher than semicircular; costal segments 3 and 4 as 0.19:0.16 ..... ***compositella* (Malloch).**

7. Post gena extending half or more than half vertical height of eye ..... 8.
- Post gena extending only 1/4th vertical height of the eye; lunule semicircular; penultimate sector of M_{1+2} about 1/7th ultimate; aedeagus with distinct hypophallus and epiphallus ..... ***bharati* Singh and Ipe**
8. Post gena extending half vertical height of eye; mesonotum equal in length and width; mediphallus and basiphallus with prominent marginal chitinization ..... ***manii* Singh and Ipe**
- Post gena extending 3/4th vertical height of eye; mesonotum longer than broad as 4.9:3.9; distiphallus with paired bulb like extremites ..... ***rosicae* sp. nov.**
9. Gena less than half vertical height of eye 10
- Gena more than 1/2 vertical height of eye; *acr* 11 arranged in one row of 4, one row of 3 and two rows of two abreast ..... ***taraia* Garg**
10. Gena 1/6 vertical height of eye 11
- Gena wider than 1/6 vertical height of eye 12
11. Ultimate sector of costa less than half the penultimate; aedeagal apodeme, hypandrium ratio 1.46:0.88; mandibles unequal, right with 3 cutting edges each ..... ***bengalensis* sp. nov.**
- Ultimate and penultimate sectors of costa more or less equal; aedeagal apodeme, hypandrium ratio 0.11:0.07; mandibles equal with two cutting edges each ..... ***ujjainensis* sp. nov.**
12. Gena 1/3rd vertical height of eye 13
- Gena less than 1/3rd vertical height of eye 17
13. *r-m* more or less at mid point of discal cell as 6:5; ultimate and penultimate sectors of costa 3.7:2.5; aedeagus long, more than half the length of aedegal apodeme ..... ***kumaonensis* Garg**
- *r-m* distinctly away from mid point of discal cell 14
14. *acr* in three rows of four abreast, ultimate and penultimate sectors of costa 11:10; hypandrium more or less 'V' shaped ..... ***sonchi* Hendel**
- *acr* in distinctly more than 3 rows either in five or six rows 15
15. *acr* 24 in five rows of 4 and two rows of 2 abreast; ultimate and penultimate sectors of costa in the ratio 1.9:1.5; *r-m* distad of mid point of discal cell as 12:5; anal developed 2/3rd its length ..... ***himalayana* Garg**
- *acr* in 6 rows 16
16. *acr* 16 arranged in six rows of 3, 2, 2, 3, 2, 4; ultimate and penultimate sectors of M_{3+4} in the ratio 3.2:1; anal developed only 1/3rd its length ..... ***kumiliensis* sp. nov.**
- *acr* 30, arranged in six rows of 5 abreast; ultimate and penultimate sectors of M_{3+4} 6.1:2.2; anal developed 2/3 its length ..... ***ornephila* Garg**
17. Gena less than 1/4th vertical height of eye 18

- Gena more than 1/4th vertical height of eye; *r-m* distad of mid point of discal cell as 14:3; ultimate and penultimate sectors of costa equal ... ... ***domestica* Garg**

18. *acr* in 8 rows of 5 abreast; *r-m* distad of mid point of discal cell as 22:78; articulating arms of hypandrium slightly broad ..... ***vandiperiyarensis* sp. nov.**

- *r-m* distad of mid point of discal cell as 57:78; ultimate and penultimate sectors of costa unequal 1.7:1.5 ..... ***idukkiensis* sp. nov.**

DESCRIPTION OF NEW SPECIES

i. *Liriomyza bengalensis* sp. nov.

Head (Fig. 1, 2): Frons slightly raised above level of eye in profile, width at vertex slightly less than twice that at mid ocular region as 1.12:0.69; parafacial area distinct, moderately broad; gena moderate, slightly less than half as long as broad as 0.035:0.085, about 1/6th vertical height of eye; eyes longer than broad as 0.24:0.16, broadly oval; *pvt* less than half the *inv* as 0.55:0.14; *ovt* more than half the *inv* as 0.07:0.14; *ors* two pairs, more or less equal; *ori* two pairs, more or less equal, ratio of upper *ors* and lower *ori* 0.086:0.074; antennal bases not approximate; lunnule higher, more or less inverted 'V'-shaped; 3rd antennal segment oval, longer than broad as 0.053:0.043; arista long, longer than width of eye as 1.83:1.66; orbital setulae reclinate.

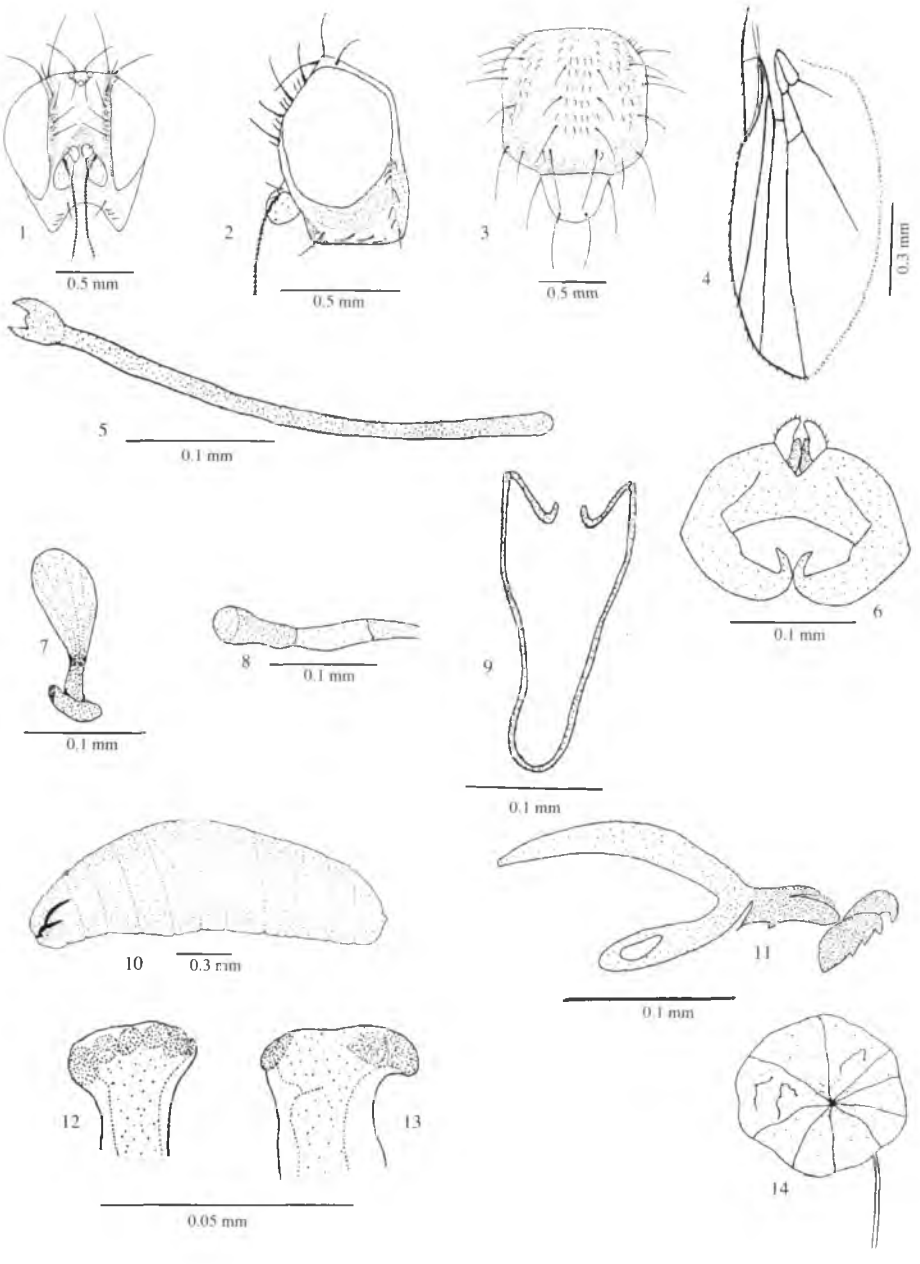
Thorax (Fig. 3): Notum longer than broad as 0.46:0.33; scutellum broadly ovate distally, slightly less than 1/3rd length of notum as 0.12:0.34; humeral callus distinct, transverse sulcus not extending on to the dorsum; *dc* four, *dc*1 longest, *dc* 1–4 in the ratio 0.18:0.15:0.096:0.068 in the ascending order; apical *sc* cruciate, apical and subapical *sc* equal; *acr* 24, in six rows of four abreast.

Wing (Fig. 4): Longer than broad as 0.69:0.28; costa extending to M_{1+2}, 1–4 costal segments in the ratio 0.23:0.29:0.12:0.10; *r-m* away from midpoint of discal cell as 0.59:0.39, $r - m$, $m - m$ ratio 0.16:0.47; ultimate and penultimate sectors of M_{1+2} in the ratio 4.19:0.39; ultimate sector of M_{3+4} almost three times the penultimate as 0.22:0.07; anal not reaching wing margin, developed only half its length.

Colour: Frons, gena, antennae yellow; ocellar triangle black; eye ferruginous; arista black; notopleural area yellow with a black spot; dorso-median region of scutellum yellow; humeral callus yellow; halters yellow.

Genetalia (Fig. 5–9): Hypandrium 'U'-shaped with no hypandrial apodeme, articulating surface not broad, slender with arm without prominent sclerotisation; aedeagal apodeme well formed, slightly less than half the hypandrial arm as 1.46:0.88; distiphallus longer than usual, basi, medi and distiphallus in the ratio 0.02:0.027:0.031; periandrium broader than long as 0.73:0.30 surstyli prominent, incurved; ejaculatory bulb well formed, more than twice as long as broad as 0.05:0.02.

Final instar Larvae (Fig. 10–13): Longer than broad as 1.18:0.33; both spiracles stalked, anterior with 6 and posterior with 3 bulbs; cephalopharyngeal skeleton well formed, mandibles longer than broad as 0.18:0.08, right mandible with three and left with two cutting edges, upper arm of paraclypeal phargma almost three times the



FIGURES 1–14. *Liriomyza bengalensis* sp. nov. 1, Head front view; 2, Head in profile; 3, Thorax; 4, Wing; 5, Aedeagal apodeme; 6, Epandrium; 7, Ejaculatory apparatus; 8, Phallus; 9, Hypandrium; 10, Final instar larva (entire); 11, Cephalopharyngeal skeleton; 12, Anterior spiracles; 13, Posterior spiracles; 14, Host plant leaf with mines (*Nasturtium* sp. F-Cruciferae).

intermediate sclerite as 0.081:0.24, lower arm slightly more than half the upper arm as 0.13:0.24, lower arm with a prominent foramen.

Biological notes (Fig. 14): The larvae make upper surface serpentine leaf mines on *Nasturtium* leaves, the pupation takes place in the soil.

Host Plant: *Nasturtium* sp. (Cruciferae)

Material studied: Holotype: ♂ on pin. Coll. Ashwani Kumar, Loc. Kalimpong (W.B.), date of collection 5.iv.2004.

Paratype: 3♂♂ and 14♀♀ on wing with collection data same as above.

Comparison: This species comes close to *Liriomyza ujjainensis* sp. nov. but can be differentiated from it with the help of ratio of costal segments.

Etymology: The name *bengalensis* is derived from West Bengal.

ii. *Liriomyza idukkiensis* sp. nov.

Head (Fig. 15, 16): Frons moderately wide at vertex, twice as wide as the mid ocular region of frons as 1.07: 0.58, projecting above and slightly in front of eye in profile; para frontalia well marked with medium sized *ors* and *ori*; eyes broad oval, longer than broad as 2.36:1.73; antennal bases approximated, 3rd segment elongated, longr than broad as 0.56:0.43; arista long almost as long as width of eye as 1.70:1.73; orbital setulae reclinate; *pvt* divergent, medium 0.75:0.94 the *inv*; *ovt* shorter than *inv* as 0.82:0.94; *ori* longer than *ors*; gena broad, half as broad as long as 0.42: 0.89, about 1/5th vertical height of the eye.

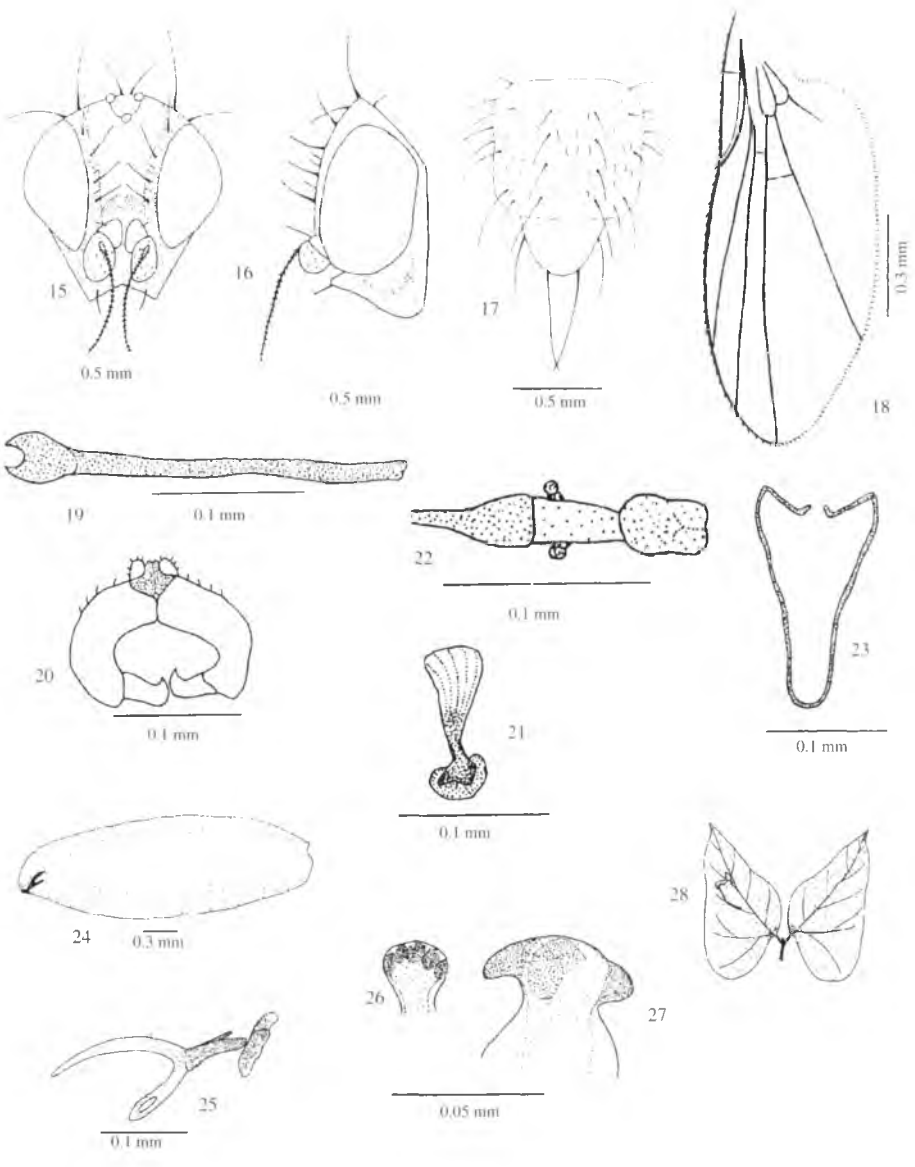
Thorax (Fig. 17): Longer than broad as 3.90:3.21; scutellum slightly more than one third the notum as 1.04: 2.86, broadly triangular apically; humeral callus distinct; *dc*4, 1.67:1.34:0.92:0.76 in the ascending order; *acr* 12, in five rows of 2,3,3,2 and 2 abreast; *h* long, longer than *dc* 4 as 1.0:0.76; apical scutellars cruciate, longer than subapicals as 1.55:1.26.

Wing (Fig. 18): Longer than broad as 8.20:3.39; costa extending upto M_{1+2}, 1–4 costal segments in the ratio 2.71:3.21:1.07:1.05; both cross veins present; *r-m* away from mid point of discal cell as 0.57:0.78; *m–m* longer than *r-m* as 0.50:0.20; ultimate and penultimate sectors of M_{1+2} in the ratio 4.60:0.57; ultimate and penultimate sectors of M_{3+4} in the ratio 2.75:1.27.

Colour: Frons, gena, antenna yellow; ocellar triangular black; eye ferruginous; arista black; notopleural area and dorso-median region of scutellum yellow, black spot on the yellow part of notopleural area; humeral callus yellow; halters yellow.

Genitalia (Fig. 19–23): Hypandrium U-shaped with no apodeme, hypandrial arms slender, articularting arms moderate, devoid of prominent sclerotized articulating surface; aedeagal apodeme long, longer than hypandrium as 1.02:0.69; distiphallus and mediphallus more or less equal as 0.17:0.16, basiphallus 0.25:0.17 the distiphallus, distiphallus bilobulate; ejaculatory bulb twice as long as wide as 0.38:0.15, with moderate scterolisation; periandrium broader than long as 0.54:0.19

Final instar (Fig. 24–27): More than 3× as long as broad as 7:2.08; both spiracles stalked, anterior with 5 and posterior with 3 spiracular bulbs; cephatopharyngeal skeleton well sclerotised, mandibular sclerite longer than broad as 0.23:0.08, each



FIGURES 15–28. *Liriomyza idukkiensis* sp. nov. 15, Head front view; 16, Head in profile; 17, Thorax; 18, Wing; 19, Aedeagal apodeme; 20, Epandrium; 21, Ejaculatory apparatus; 22, Phallus; 23, Hypandrium; 24, Final instar larva (entire); 25, Cephalopharyngeal skeleton; 26, Anterior spiracles; 27, Posterior spiracles; 28, Host plant leaf with mines (*Dolicus lablab* F-Leguminosae).

with two cutting edges, intermediate sclerite with a clear basal break, less than 1/3rd the dorsal arm of cephalopharyngeal skeleton as 0.10:0.26; lower arm slightly longer than intermediate sclerite as 0.12:0.10, lower arm with distinct foramen.

Biological notes: Larva makes a linear, irregular upper surface mine on the host plant *Dolicus lablab* (Fig. 28). Pupation takes place mostly in the soil.

Host plant: *Dolicus lablab* (Leguminosae)

Material studied: -Holotype: ♂ on pin. Coll. Ashwani Kumar, Loc. Kumili, Distt.–Idukki (Kerala), date of collection 25.xii.2003.

Paratypes: 1♂ and 2♀♀, Collection data same as above.

Comparison : This species comes close to *Liriomyza vandiperiyarensis* sp. nov

Etymology: The name *idukkiensis* derived from Idukki, the district in Kerala from where the collection was made.

iii. *Liriomyza kalimpongensis* sp. nov.

Head (Fig. 29, 30): Frons not projecting above or in front of eye in profile, at vertex wider than at mid ocular region as 0.95:0.70; parafrontalia narrow; *ors* and *ori* two pairs each, upper *ori* 0.98:0.77 the lower *ors*; *pvt* divergent, almost half as long as *inv* as 0.76:1.39; *ovt* and *inv* 0.90:1.39; antennal bases not approximate; lunule high, more or less acutely triangular; eyes longer than broad as 0.95:0.58; gena broad, two thirds as long as wide as 0.60:0.90; 3rd antennal segment longer than broad as 0.065:0.050; arista long, more than twice the length of eye as 1.97:0.95; post gena extending marginally behind eye; eyes more or less quadrate.

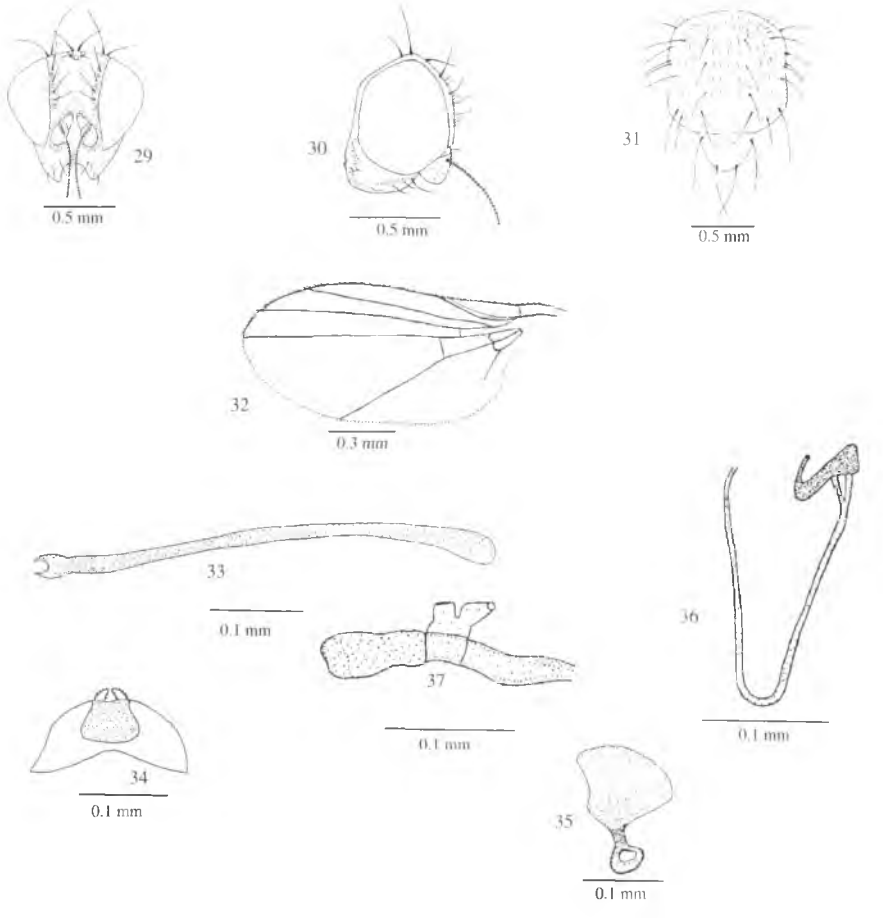
Thorax (Fig. 31): Longer than wide as 4.55:3.27; sculellum almost one-third the length of dorsum as 1.21:3.34; humeral callus distinct; *dc* four in the ratio of 1.98:1.62:1.11:0.78 in ascending order; *h* longer than *dc*4 as 0.93:0.78; apical *sc* cruciate, shorter than sub apicals as 1.56:1.63; *acr* 20, arranged in 5 rows of 4 abreast.

Wing (32): Longer than broad as 0.87:0.38; costa extending to M_{1+2}, 1–4 costal segments in the ratio 0.27:0.40:0.13:0.13; ultimate and penultimate sectors of costa equal; both cross veins present; *r-m* away from mid point of discal cell as 0.085:0.057; *r-m* less than one third the *m-m* as 0.020:0.055; ultimate and penultimate sectors of M_{1+2} in the ratio 6.09:0.85; ultimate and penultimate sectors of M_{3+4} in the ratio 0.34:0.13, anal not reaching the wing margin, developed about half its length.

Colour: Frons, gena, antenna yellow; ocellar triangular black; eye ferruginous; arista black; noto-pluera and dorso median region of scutellum yellow with black spot on notopleural area; humeral callus yellow; halters yellow.

Genetalia (Fig. 33–37): Hypandrium broadly 'U'-shaped, hypandrial apodeme absent, hypandrial arms moderately sclerotised, articulating surface broad; aedeagal apodeme long; more than twice the hypandrial arms as 0.20:0.09; aedeagus with basal and distal region more or less equal as 0.035:0.032, mediphallus less than half the distiphallus as 0.14:0.32; ejaculatory bulb distinct, large, fan-shaped, almost as broad as long 0.052:0.65; periandrium broad, broader than long as 0.73:0.28.

Material studied: Holotype: ♂ on wing. Coll. Ashwani Kumar, Loc. Kalimpong (W.B.), date of collection 6.iv. 2004.



FIGURES 29–37. *Liriomyza kalimpongensis* sp. nov. 29, Head front view; 30, Head in profile; 31, Thorax; 32, Wing; 33, Aedeagal apodeme; 34, Epandrium; 35, Ejaculatory apparatus; 36, Hypandrium; 37, Phallus.

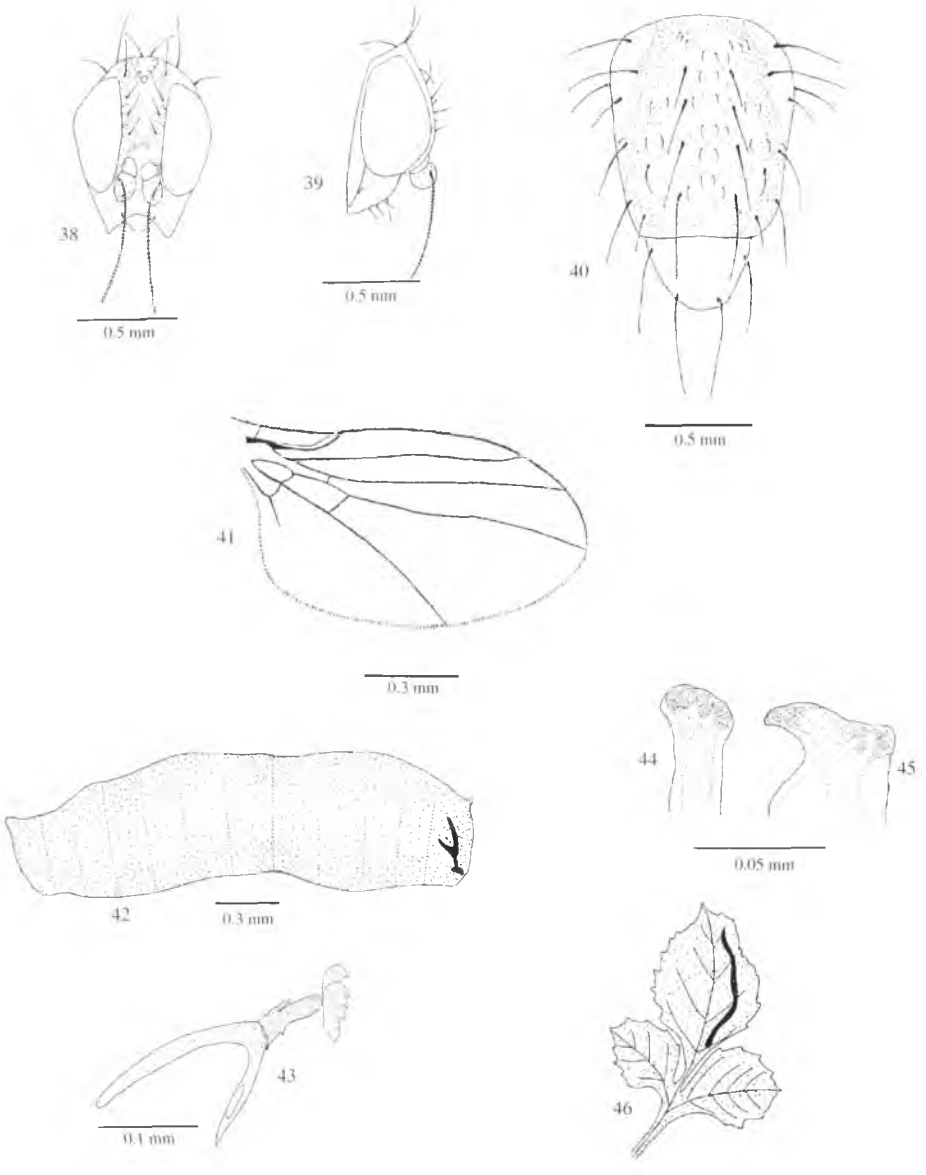
Paratype: 4♂♂ and 14♀♀ on wing, collection data same as above.

Comparison: This species comes close to *Liriomyza alticola* Garg but can be differentiated by the differences in the arrangement of the *acr*.

Etymology: The name is derived from Kalimpong, the place of collection.

iv. *Liriomyza kumiliensis* sp. nov.

Head (Fig. 38, 39): Almost as broad as high, frons moderately wide, less than the vertical height of eye as 0.78:1.90, projecting above level of eye in profile, narrow medially less than half the width of upper frons as 0.28:0.78; eyes half as wide as high



FIGURES 38–46. *Liriomyza kumiliensis* sp. nov. 38, Head front view; 39, Head in profile; 40, Thorax; 41, Wing; 42, Final instar larva (entire); 43, Cephalopharyngeal skeleton; 44, Anterior spiracles; 45, Posterior spiracles; 46. Host plant leaf with mines.

as 0.95:1.90; gena moderate, slightly more than 1/3rd the vertical height of the eye as 0.61:1.90; third antennal segment oblong, longer than wide as 0.47:0.37; arista long, 1.35:1.90 the height of eye; *ovt* and *inv* more or less equal; *pvt* slightly shorter than *inv* as 0.65:0.69; orbitals and orbital setulae reclinate, *ors* 0.58:0.56 and *ori* 0.43:0.36.

Thorax (Fig. 40): Longer than wide as 3.50:2.60; Scutellum moderate, 0.10:0.26 the dorsum; *dc* 4, *dc* 1 longest, *dc* 1–4 in the ratio 1.40:1.00:0.52:0.32 in ascending order; *acr* 6 rows of 3, 2, 2, 3, 2, 4 abreast; apical scutellar long, 14:11 the prescutellar; h prominent, twice as long as *dc* 4 as 0.66:0.32.

Wing (Fig. 41): longer than wide as 7.90:3.50; casta extending upto M_{1+2}, 1–4 costal segments in the ratio 2.70:3.30:1.15:1.20; both cross veins present; *r-m* 0.19:0.44 the *m-m*, *r-m* away from midpoint of discal cell as 0.70:0.50; ultimate and penultimate sectors of M_{3+4} as 3.20:1.0; ultimate and penunltimate sectors of M_{1+2} as 5.09:0.46; anal not reaching wing margin, developed less than 1/3rd its length.

Colour: Frons, gena, antenna, yellow, ocellar triangular black; eye ferruginous; arista black; humeral callus yellow; notopleural area and upper surface of scutellum yellow; Halters yellow.

Final instar (Fig. 42–45): Longer than broad as 3.50:1.0; both spiracles present, stalked; anterior spiracles with 5dl bulbs and posterior spiracle with 3 bulbs; cephalopharyngeal skeleton prominent; mandibles with 2 cutting edges, longer than broad as 2.10:1.10; intermediate sclerite moderate, 1.0:1.30 ratio of upper arm of paraclypeal phragma; upper and lower arms of paraclypeal phragma in the ratio 2.20:1.30.

Biological notes: The larvae make linear mines (Fig. 46), frass deposition in an irregular fashion, the last instar larva falls into the soil for pupation.

Host Plant: Unidentified flowering, seasonal, ornamental plant.

Material Examined: Holotype: ♂ on pin. Coll. Ashwani Kumar, Loc. Kumili, Distt. - Idukki (Kerala) date of collection 27. xii. 2003.

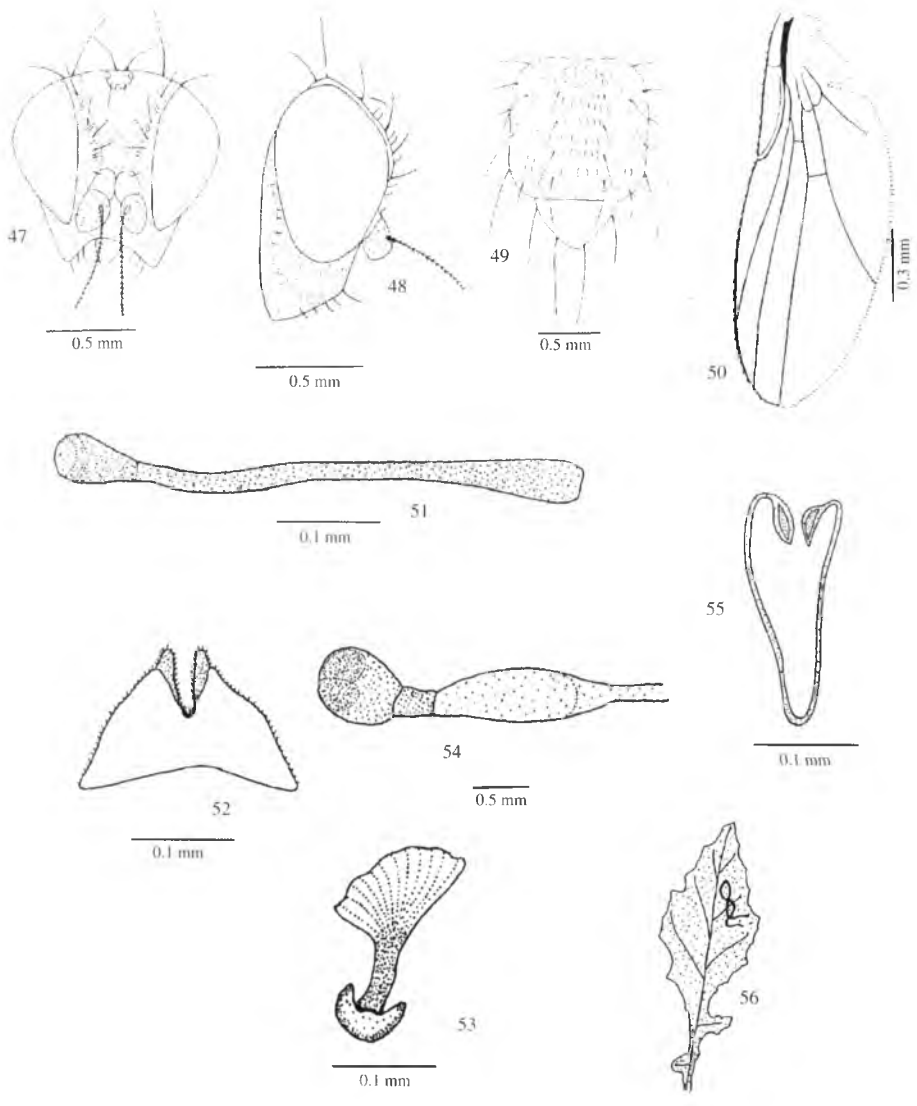
Paratypes: 1♂ and 7♀♀, collection data same as above.

Comparison: This species comes close to *Liriomyza ornephila* Garg but can be differentiated from the former by the difference in the arrangement of *acr*, and relative length of anal vein.

Etymology: The name *kumiliensis* is derived from Kumili, the place of collection.

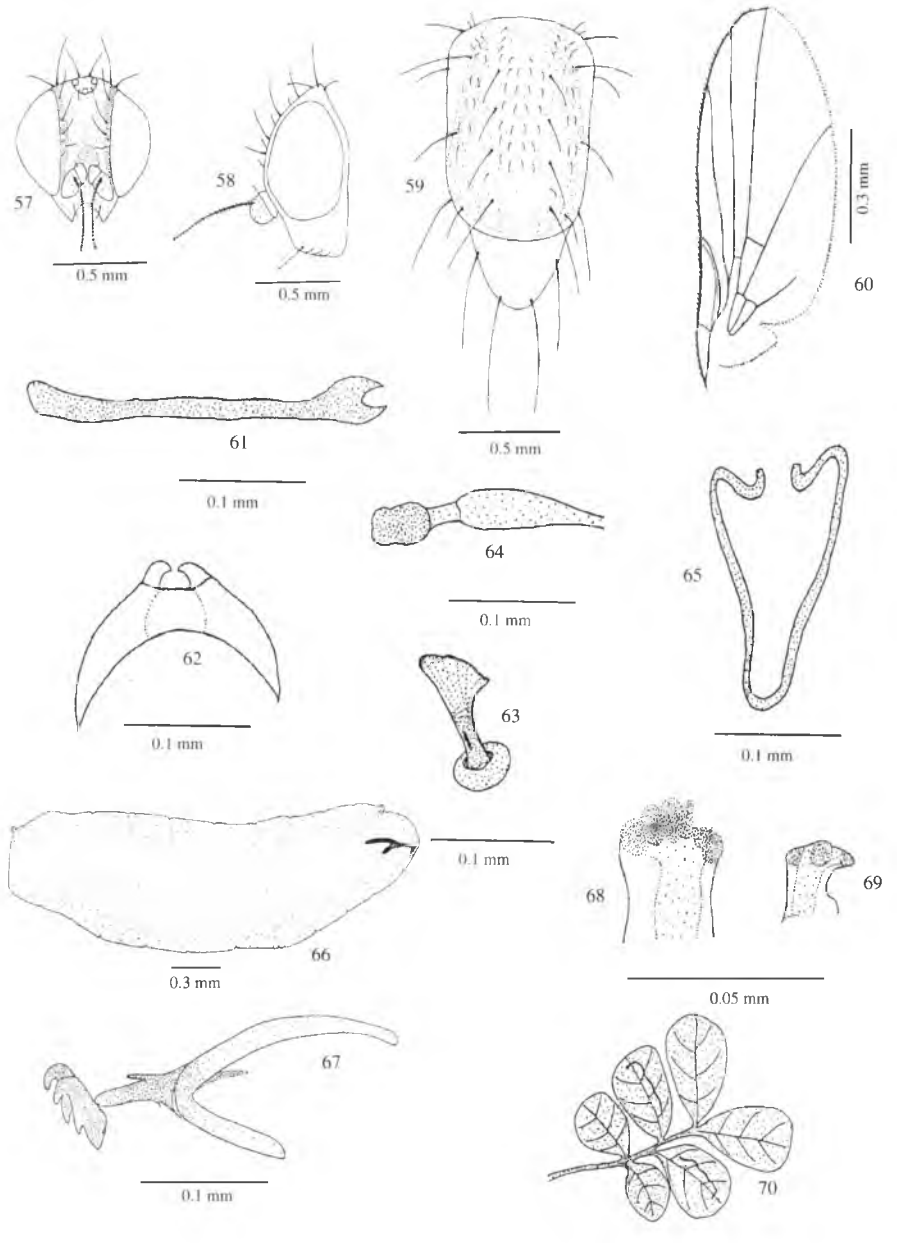
v. *Liriomyza rosicae* sp. nov.

Head (Fig. 47, 48): Frons broad, slightly projecting above and more or less at level with eye in profile, vertical region broad broader than intra ocular region as 1.25:0.70, parafrontalia broad, broadest at mid intra ocular region; eyes longer than broad as 2.41: 1.86; antennal bases approximate; *pvt* about two-thirds the *inv* as 0.85:1.18; *ovt* shorter than *inv* as 0.81:1.18; orbital setulae reclinate; 3rd antennal segment longer than broad as 0.80:0.58; arista more than twice the third antennal segment; gena broad, more than half as long as 0.63:0.11, about 1/4th vertical height of the eye; lunule more or less inverted 'Λ'-shaped.



FIGURES 47–56. *Liriomyza rosicae* sp. nov. 47, Head front view; 48, Head in profile; 49, Thorax; 50, Wing; 51, Aedeagal apodeme; 52, Epandrium; 53, Ejaculatory apparatus; 54, Phallus; 55, Hypandrium; 56, Host plant leaf with mines (*Sonchus* sp. F-Compositae).

Thorax (Fig. 49): Longer than broad as 4.97:3.90; scutellum broad at base, slightly less than one-third the length of dorsum; humeral callus distinct with moderately longer *h*; *dc* four, 1.81:1.27:1.0:0.89 in the ascending order; *acr* numerous, about 36 arranged in six irregular rows of 6 abreast.



FIGURES 57–70. *Liriomyza ujjainensis* sp. nov. 57, Head front view; 58, Head in profile; 59, Thorax; 60, Wing; 61, Aedeagal apodeme; 62, Epandrium; 63, Ejaculatory apparatus; 64, Phallus; 65, Hypandrium; 66, Final instar larva (entire); 67, Cephalopharyngeal skeleton; 68, Anterior spiracles; 69, Posterior spiracles; 70, Host plant leaf with mines (*Cassia tora* Linn. F-Leguminosae).

Wing (Fig. 50): Longer than broad as 8.83:4.43; costa extending upto M_{1+2}, 1–4 costal segments in the ratio of 3.08:3.90:1.32:1.03; both cross-veins present; *r-m* before mid point of discal cell as 0.58:0.80, *r-m*, *m-m* ratio 0.26:0.60; penultimate and ultimate sectors of M_{1+2} as 0.80:5.92; penultimate and ultimate sectors of M_{3+4} 1.43:3.16; anal not reaching wing margin, developed more than two-thirds its length.

Genitalia (Fig. 51–55): Hypandrium 'U'-shaped with no hypandrial apodeme, hypandrial arms with articulatory surface broad, half as broad as long as 0.50:1.0; aedegal apodeme well sclerotised, twice as long as hypandrium as 1.42:0.76; basiphallus long; distiphallus with paired bulb-like extremities, mediphallus short, half as long as distiphallus as 0.10:0.22; ejaculatory bulb prominent, 0.35:0.43 as broad as long; periandrium broader than long as 0.65:0.32, terminally bilobate and triangular.

Colour: Frons, gena, antennae yellow; ocellar triangular black; eye ferruginous; arista black; notopleural areas and upper surface of scutellum yellow; humeral callus yellow; halters yellow.

Biological observations: The immature stage makes linear mines on upper surface of leaves (Fig. 56), frass deposition in irregular fashion. The last instar larvae fall into the soil for pupation.

Host plant: *Sonchus sp.* (Compositae)

Material studied: Holotype: ♂ on pin. Coll. Ashwani Kumar, Loc. Vandiperiyar, Distt.- Idukki (Kerala) date of collection 25.xii.-2003.

Paratype: 5♂♂ on wing and 3♀♀ on pin, collection data same as above.

Comparison: This species comes close to *Liriomyza manii* Garg but can be separated from the former by the length of gena and ratio of length and width of mesonotum.

Etymology: The species is named after the Ist author's mother Rosie who stood by him during the course of this work.

vi. *Liriomyza ujjainensis* sp. nov.

Head (Fig. 57–58): Frons projecting marginally above the eye in profile, in level with the eye in front, mid ocular region 3/4th the vertical region as 068:0.96; parafacials moderately broad; gena more than one-third as broad as long as 0.043:0.12, about 1/6th vertical height of eye; post gena not extending behind eye; eyes longer than broad as 0.22:0.18; antennal bases more or less approximate; lunule moderately high, more or less in the shape of inverted 'V'; 3rd antennal segment longer than broad as 0.073:0.051; arista less than width of the eye as 0.15:0.18, *pvt* divergent, equal to *ovt* as 0.80:0.81 *inv* and *ovt* in the ratio of 1.10:0.81; orbital setulae reclinate.

Thorax (Fig. 59): Longer than broad as 0.43:0.35; scutellum broadly ovate distally; transverse sulcus not extending on to the dorsum; humeral callus distinct; *dc* four, *dc*1 longest, *dc* 1–4 in the ratio 0.17:0.13:0.07:0.06 in the ascending order; prescutellars and scutellars equal; *acr* 24 in 6 rows of 4 abreast.

Wing (Fig. 60): Longer than broad as 0.80:0.35; costa extending upto M_{1+2}, ultimate and penultimate sectors of costa equal, 1–4 costal segments in the ratio of 0.24:0.35:0.19:0.19; both cross-veins present; r-m distad of mid point of discal cell as 0.073:0.057, *r-m*, *m-m* ratio 0.20:0.60; ultimate and penultimate sectors of M_{1+2}

in the ratio of 5.65:0.73; ultimate and penultimate sectors of M_{3+4} in the ratio of 0.32:0.12; anal not reaching wing margin, developed less than 2/3rd its length.

Colour: Frons, gena, antennae yellow; ocellar triangular black; arista black; eye ferruginous; notoplural yellow with a black spot, dorsal region of scutellum yellow; humeral callus yellow; halters yellow.

Genitalia (Fig. 61–65): Hypandrium 'U'-shaped with no hypandrial apodeme; articulating arms not well sclerotised, sigmoid; length of aedeagal apodeme and hypandrium in the ratio 0.11:0.07; basiphallus long, mediphallus shorter than distiphallus as 0.018:0.016; ejaculatory apparatus longer than broad as 0.037:0.018, bulbous region comparatively prominent; periandrium broader than long as 0.06:0.018.

Larva (Fig. 66–69): Final instar larva longer than broad as 1.58:0.44; spiracles stalked, anterior with 6 and posterior with 3 bulbs; cephalopharyngeal skeleton well sclerotised, mandibles equal, with two cutting edges each, dorsal arm and intermediate sclerite in the ratio of 2.30:0.90, ventral arm more than double the intermediate sclerite as 0.09:0.12, no foramen on the ventral arm.

Biological notes: Immature makes linear serpentine upper surface mines on *Cassia tora*, leaves (Fig. 70) with serrated margin and apposite arrangement, pupation takes place in the soil.

Host Plant: *Cassia tora* Linn. (Leguminosae)

Material studied: Holotype: ♂ on pin. Coll. Ashwani Kumar, Loc. Rishinagar, Distt. - Ujjain (M.P.), date of collection 11.viii.2004.

Paratypes: 2♂♂ and 12♀♀ on pin, and 3♂♂ on wing. Coll. Ashwani Kumar, Loc. Rishinagar, Distt. - Ujjain (M.P.), date of collection 12.viii.2004.

Comparison: This species come close to *Liriomyza bengalensis* sp. nov. but can be separated with the help of relative ratios of costal segments of both.

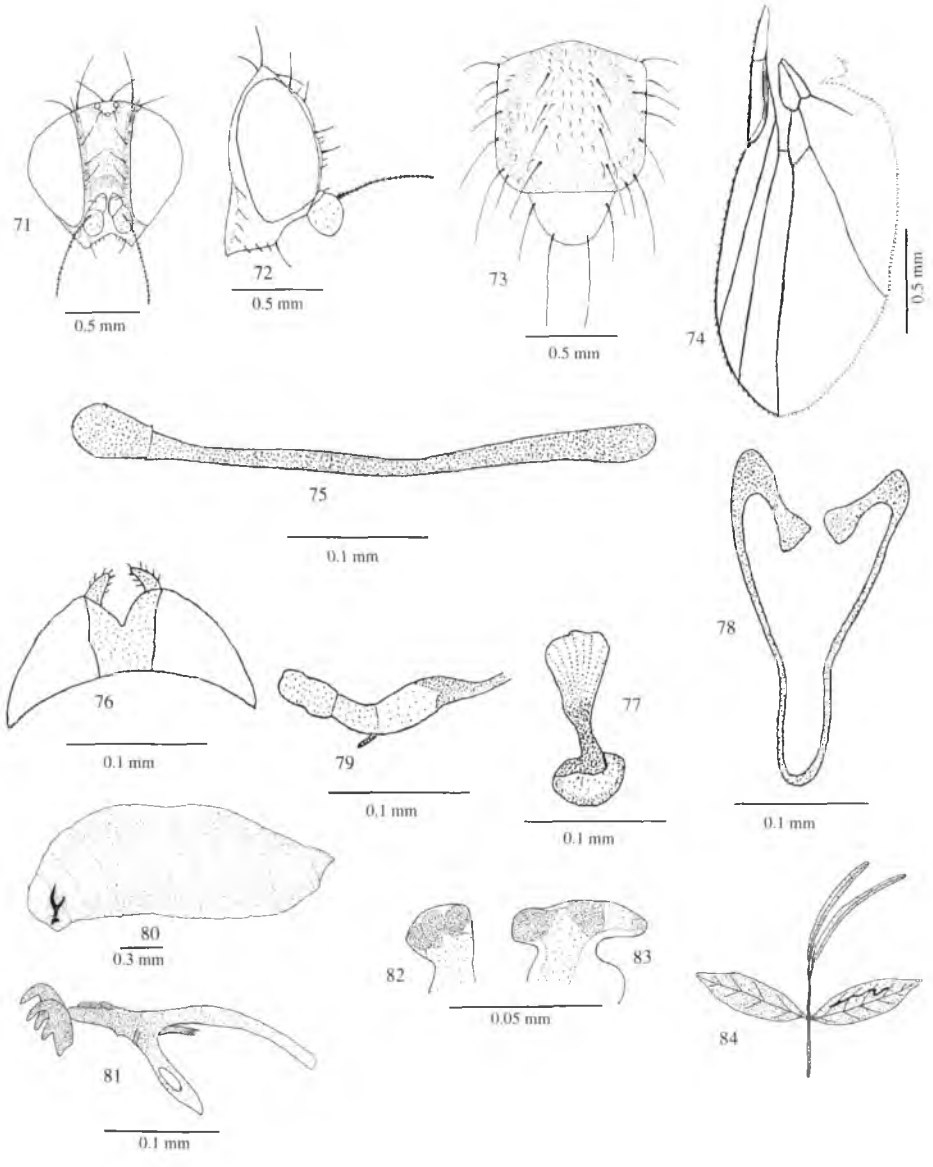
Etymology: The name *ujjainensis* is derived from Ujjain, the district in Madhya Pradesh from where the collection was made.

vii. *Liriomyza vandiperiyarensis* sp. nov.

Head (Fig. 71,72): Frons at vertex wide, its width at vertex and inter orbital region in the ratio 0.11:0.08, , projecting slightly above and in front in profile; eyes oblong, longer than wide as 0.25:0.18; gena 1/5th vertical height of eye, moderately wide, less than half as long as 0.50:1.10, postgena extending behind eye above; *pvt* shorter than *inv* as 0.72:0.97; *ovt* shorter than *inv* as 0.90:0.97; orbitals 4, reclinate; orbital setulae present, reclinate; third antennal segment longer than wide as 0.82:0.55; arista long, thrice as long as third antennal segment as 2.55:0.82, equal to the length of eye.

Thorax (Fig. 73): Longer than broad as 4.26:3.63; scutellum broadly ovate distally, less then 1/3rd the length of dorsum; *dc* 4, prominent, long 1.43:1.30:1.02:0.84 in the ascending order; *acr* present in 8 not so regular rows of 5 abreast; h shorter than *sa* as 1.00:1.20, apical sc longer than sub apicals as 1.40:1.35.

Wing (Fig. 74): Longer than braod as 7.08:3.59; costa extending upto M_{1+2}, 1–4 costal segments in the ratio 2.35:2.28:1.05:1.06; both cross veins present; *r-m*, *m-m* ratio 0.16:0.43, *r-m* distad of midpoint of discal cell as 0.78:0.22; penultimate and



FIGURES 71–84. *Liriomyza vandiperiyarensis* sp. nov. 71, Head front view; 72, Head in profile; 73, Thorax; 74, Wing; 75, Aedeagal apodeme; 76, Epandrium; 77, Ejaculatory apparatus; 78, Hypandrium; 79, Phallus; 80, Final instar larva (entire); 81, Cephalopharyngeal skeleton; 82, Anterior spiracles; 83, Posterior spiracles; 84, Host plant leaf with mines (*Cassia* sp. Linn. F-Leguminosae).

ultimate sectors of M_{1+2} in the ratio of 0.22:4.44; penultimate and ultimate sector of M_{3+4} in the ratio of 0.83:2.63; anal not reaching wing margin, developed about half its length.

Colour: Frons, gena yellow; antenna yellow; ocellar triangular black; eye ferruginous; arista black; notopleural areas and upper surface of scutellum yellow, one black spot on yellow patch of notopleural area, rest of dorsum black, humeral callus yellow; halters yellow.

Genitalia (Fig. 75–79): Hypandrium 'U'-shaped, without apodeme, hypandrial arms broad distally, 0.90:0.49 as long as wide, hypandrial arms incurved, articulating surface slightly broad; aedeagal apodeme slightly less than twice the hypandrium as 1.63:0.90; basi, medi and distiphallus regions distinct, basiphallus longer than mediphallus and distiphallus as 0.39:0.15:0.17; ejaculatory pump distinct, longer than broad as 0.46:0.20.

Larva (Fig. 80–83): Final instar larva longer than wide as 10.0:3.66; segmental boundaries distinct; both the spiracles with short stalks, three spiracular bulbs on each; cephalophryngeal skeleton moderately sclerotised; mandible longer than broad as 0.20:0.14, each mandible with true cutting edges, intermediate sclerite with partial break, dorsal arm of paraclypeal pharagma more than twice the intermediate sclerite as 2.40:1.04, lower arm slightly more than half the upper arm as 1.32:2.40, lower arm with a foramen.

Material studied: Holotype: ♂ on pin. Coll. Ashwani Kumar, Loc. Vandiperiyar, Distt.- Idukki (Kerala), date of collection 25.xii.2003.

Paratypes: 16♂♂ and 29♀♀, collection data same as above.

Biological notes: immature stages make linear mines on the upper surface of leaves (Fig. 84), frass deposition in irregular fashion. The last instar larva falls into the soil for pupation.

Host plant: *Cassia* sp. (Leguminosae)

Comparison: This species comes close to *Liriomyza idukkiensis* sp. nov. but can be differentiated bydifferences in the arrangement of *acr*.

Etymology: The species name is derived from the place of collection.

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REFERENCES

Garg P. K. (1971) Studies on Agromyzidae (Diptera) from the Gangetic Basin. Part II. Oriental Insects (Suppl.), 1: 185–212; Part III. 1: 213–234; Part IV. 1: 235–246; Part V. 1: 247–256; Part VI. 1: 257–266; Part VII. 1: 267–282.

Garg P. K. (1971) Studies on Agromyzidae (Diptera) from the Gangetic Basin. Part I. Descriptions of three new species. Oriental Insects, 5(2): 179–188.
Singh S. and Ipe M. Ipe (1973) The Agromyzidae from India. Memoirs of the School of Entomology, 1: 1–286.

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Distribution of eggs and larvae of *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae) in relation to different plant parts

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ABSTRACT: *Leucinodes orbonalis* is the predominant pest of brinjal. A study was carried out to determine the distribution of eggs and larvae in relation to various plant parts under field conditions as this knowledge is useful for successful management of the borer. Results indicated that 55% of the eggs are laid on lower surface of leaf followed by 38% on flower and 7% on fruits. About 5–28% of the flower buds harbored initial larval stages of the insect.

KEYWORDS: brinjal, *Leucinodes orbonalis*, egg, larva, distribution

Eggplant, *Solanum melongena* L. is an important vegetable of South Asian countries, contributing to 26 percent of the world produce. The shoot and fruit borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae) is the predominant pest (Ahmad, 1977) causing up to 80% yield loss. The damage is inflicted initially on the shoots prior to flowering and later on the fruits.

One of the factors that aids in successful management of the pest is knowledge on the occurrence of the susceptible stages of the pest in relation to various plant parts. Perusal of literature indicated lack of adequate information on the distribution of eggs and larval stages in relation to different parts of the plant such as shoots, leaves, fruits and flowers under field conditions. A study was therefore carried out to determine the distribution of these stages on the plant.

The study was carried out at Indian Institute of Horticultural Research Farm, Bangalore, in an area of 500 sq. m., on brinjal var. Arka Neelakanth, a borer susceptible variety. Observations were initiated with the incidence of borer damage on shoots. For recording distribution of eggs on the plant, terminal shoot tips (10 cm length), leaves (top eight leaves), buds, flowers and fruits (2–3 cm long) were collected randomly from the plants. Fruits of 1–2 cm length were also collected randomly. Twenty five samples of each part were collected at weekly interval. Observations

*Corresponding author

on number of eggs laid in the above plant parts were recorded by checking the samples under a stereo microscope in the laboratory. For recording larval incidence on flowers, flowers of three stages (buds, half opened and fully opened flowers) were randomly collected at weekly interval. Twenty five samples of each stage were collected separately, brought to the laboratory.and observations on presence of larva and stage of larva were recorded.

Among the various plant parts observed, no eggs were found on terminal shoots, 55% of eggs were recorded on leaves, 38% on flower buds and 7% on fruits. Eggs were laid singly on the lower surface of leaves, mostly attached to veins. In flowers and fruits, eggs were laid singly on the calyx region. Of the different stages of flowers observed (buds, half opened flowers and fully opened flowers), larva was recorded on buds only. A mean of 18% of buds (range 5–28%) were infested by larvae. The larval occurrence ranged from 5 to 7% in early stage of flowering. At peak flowering, 28% of buds were infested. Only early stage (first and second larval instars) was recorded on buds. Earlier study by Patnaik (2000) had shown 29–68% larval incidence on flower buds.

The above observations could form an important aid in management of *L. orbonalis* on brinjal. Since eggs and early larvae were recorded on leaves and buds, these plant parts must be targeted for successful management of the pest by biological, chemical and botanical means.

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REFERENCES

Ahmad R. (1977) Studies on the pests of brinjal and their control with special reference to fruit borer, *Leucionodes orbonalis* Guenee (Pyralidae: Lepidoptera). Entomologist's Newsletter, 7: 2–3.

Patnaik H P (2000) Flower and fruit infestation by brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen. - damage potential vs. weather. Vegetable Science, 27: 82–83.

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Postembryonic development and reproductive behaviour of *Coranus spiniscutis* Reuter (Hemiptera: Reduviidae) in three temperature regimes

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ABSTRACT: The postembryonic development and reproductive behaviour of *Coranus spiniscutis* Reuter (Hemiptera: Reduviidae) were observed at three temperature regimes (25–30 °C, 20–25 °C, and 15–20 °C) at Gorakhpur in northern India. The postembryonic developmental period, pre-oviposition period, incubation period and duration of nymphal instars decreased while fecundity increased with increasing temperature. The temperature regime of 25–30 °C was highly suitable for the early start and successful completion of reproductive behaviour. Prolongation of mating period and reduction of successful matings were observed when the bugs were transferred from 25–30 °C to 20–25 °C and 15–20 °C.

KEYWORDS: development, reproduction, reduviid, temperature effects

Temperature plays a vital role in regulating the activities of animals and has an important quantitative effect on insect development (Isenhour and Yeargan, 1981). Over-wintering, a typical feature in the life cycle of many insects in temperate zones allows organisms to synchronize their reproductive or developmental cycle with predictably changing environment; by suppression of growth, development and reproduction to promote survivorship (Danks, 1987; Ruberson *et al.*, 1998). Over-wintering of multivoltine reduviids occurs in adult stages and is characterized by reproductive arrest and this may be particularly relevant for reduvids with relatively short generation times (Butler, 1924; Parker, 1972). An attempt was made to find out the effect of onset of winter temperature on the biology and mating behaviour of a multivoltine reduviid, *Coranus spiniscutis* Reuter (Hemiptera: Reduviidae), an effective insect predator under laboratory conditions.

A culture of *C. spiniscutis* was maintained in the laboratory under optimal conditions on rice moth larvae, *Corcyra cephalonica* Staintion. Eggs laid in the laboratory were incubated in small plastic containers with moist cotton swabs. The

TABLE 1. Effect of temperature on the biological parameters and mating behaviour of *C. spiniscutis*

Parameter	Temperature regime		
	25–30 °C	20–25 °C	15–20 °C
Biology			
Incubation period (d)	4.66 ± 0.77^{a}	8.17 ± 2.14^{ab}	19.39 ± 4.32^{bc}
Nymphal duration (d)			
I instar	3.9 ± 0.58^{a}	7.74 ± 1.63^{b}	16.81 ± 3.18^{c}
II instar	4.15 ± 0.60^{a}	8.17 ± 1.98^{ab}	19.57 ± 3.03^{c}
III instar	3.89 ± 0.75^{a}	6.95 ± 1.16^{ab}	14.80 ± 3.43^{bc}
IV instar	4.34 ± 0.70^{a}	8.33 ± 2.56^{ab}	17.41 ± 4.11^{bc}
V instar	6.36 ± 0.70^{a}	4.26 ± 0.92^{ab}	9.88 ± 3.75^{b}
Total developmental period (d)	22.64 ± 9.41^{a}	35.45 ± 8.25^{ab}	78.47 ± 17.50^{b}
Adult longevity (d)			
Male			
Female	74.52 ± 4.38^{a}	87.04 ± 11.88^{a}	117.41 ± 18.69^{a}
Pre-oviposition period (d)	83.72 ± 2.45^{a}	98.62 ± 14.78^{a}	119.86 ± 16.50^{a}
Oviposition period (d)	6.20 ± 0.93^{a}	14.54 ± 1.93^{b}	34.14 ± 9.82^{c}
Total number of egg laid	60.32 ± 2.52^{b}	46.13 ± 4.68^{b}	23.42 ± 4.38^{a}
Mating behav. (Duration in min)	173.72 ± 11.67^{c}	62.49 ± 10.37^{b}	24.32 ± 4.57^{a}
Arousal			
Approach	0.51 ± 0.10^{a}	2.55 ± 1.14^{b}	2.81 ± 0.71^{b}
Riding over	8.24 ± 3.62^{a}	26.13 ± 6.14^{b}	29.66 ± 7.92^{b}
Genitalia extension and connection	17.12 ± 3.88^{a}	31.92 ± 13.46^{ab}	48.83 ± 16.52^{b}
Copulation	0.52 ± 0.24^{a}	3.02 ± 1.83^{b}	3.18 ± 2.11^{b}
Post copulatory behaviour	88 ± 0.44^{a}	109 ± 11.35^{a}	117.38 ± 18.45^{a}
Escaping (No)	5.04 ± 0.77^{a}	7.40 ± 2.71^{a}	7.95 ± 2.62^{a}
	14.24 ± 3.75^{b}	9.46 ± 1.59^{ab}	7.45 ± 2.33^{a}

In a row, mean values followed by similar letters in superscripts are not significantly different ($P \leq 0.05$) by DMRT.

eggs were exposed to temperature regimes of 25–30 °C, 20–25 °C and 15–20 °C on different occasions. The study was made during the winter period from October to December and the desired temperature ranges were maintained by using room heaters (Lexus FH 423) as required. Data on different development parameters (Table 1) were recorded and subjected to relevant statistical analysis.

Male and female pairs of *C. spiniscutis* were kept in plastic containers for mating under the above temperature regimes. The time taken for the sequential acts of mating behaviour viz. arousal, approach, riding over, genitalia extension and connection, copulation, post-copulatory behaviour and escaping were recorded. Fifteen replicates were set up in each temperature range and the data were subjected to statistical analysis (Daniel, 1999).

BIOLOGY

The temperature regime of 25–30 °C was highly suitable for the development of *C. spiniscutis* (Table 1). The incubation period of the egg was extended as the temperature decreased from 25–30 °C to 15–20 °C. Similar results were reported in a reduviid bug, *Amphibolus venator* (Nishi and Takahashi, 2002) and a joppeicid bug, *Joppeicus paradoxus* (Morimoto *et al.*, 2007). De Clercq and Degheele (1992) reported that egg development of a pentatomid bug, *Podisus sagitta* (Fab.) ceased when they were transferred from 23 °C to below 10 °C. The nymphal duration was lowest at the higher temperature regime than the other two lower temperature regimes. The total developmental period increased from 22.64 to 78.47 days, as the temperature decreased from 30–25 °C to 20–15 °C. The effect of temperature on the developmental period was highly significant statistically (Table 1), which indicated that the developmental period of *C. spiniscutis* depended mainly on the prevailing temperatures. High temperature accelerated the growth thereby shortening the developmental period (Ali and Watson, 1978; Nishi and Takahashi, 2002; Isenhour and Yeargan, 1981). According to Chapman (1971), the normal increase in metabolic rate with increasing temperature is reflected in an increased rate of development. Adult longevity both in male and female were short at 25–30 °C (Table 1). Ali and Watson (1978) reported that the greatest adult longevity in a reduviid, *Zelus renardii* was attained at 25 °C. In contrast, Morimoto *et al.* (2007) stated that longevity of cold treated bugs was generally lower than untreated ones. The Pre-oviposition period decreased with increasing temperature (Table 1). Similarly, De Clercq and Degheele (1993) reported that pre-oviposition time increased in cold storage individuals. Fecundity was adversely affected by changing temperature (Table 1). The average number of eggs laid per ovipositing female tended to increase with increasing temperature. Low temperature arrested development of eggs too, with no eggs hatching even after 30 days. Oviposition period and total number of eggs laid were maximum at higher temperature and minimum at low temperature. Rahman *et al.* (2007) reported that the female of *Xylocoris flavipes* (Hemiptera: Anthocoridae) laid maximum eggs at higher temperature and lesser at lower temperature, in agreement with the present observations. De Clercq and Degheele (1993) found that when eggs of two pentatomid bugs were transferred from 23 °C to below 10 °C the development ceased and egg survival was low, but it did not have any negative effect on survival, post storage longevity and oviposition.

REPRODUCTIVE BEHAVIOR

The temperature regime of 25–30 °C was highly suitable for the early start and successful continuation of reproductive behaviour in *C. spiniscutis* (Table 1). Similar results were reported by Usharani (1992) in a pentatomid bug, *Eocanthecona furcellata*. Duration of mating period was prolonged when the bugs were transferred from higher to lower temperature regime (Table 1). Arousal, approach, riding over, genitalia extension and connection durations were lower at 25–30 °C regime than others and the durations in other two regimes were on par. The duration of copulation and

post-copulation were on par in all temperature regimes. The escaping incidents were higher at 25–30 °C than at the two lower temperature regimes. Bose (1949) reported that adult *C. spiniscutis* was found in the field only in autumn and started to hibernate for over wintering in the litter microhabitat. Studies on the effect of winter temperature on field survival, development and sexual life of Indian reduviids are wanting; this is the first effort towards this direction.

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REFERENCES

Ali A-S. A. and Watson T. F. (1978) Effect of temperature on development and survival of *Zelus renardii*. Environmental Entomology, 7(6): 889–890.

Bose B. A. (1949) On the biology of *Coranus spiniscutis* Reuter, an assassin bug. Indian Journal of Entomology, 11: 203–208.

Butler E. A. (1924) Biology of British Hemiptera: *Coranus subapterus* Deheer. Entomologist Monthly Magazine. London, 87: 242–247.

Chapman R. F. (1971) *The insects: Structure and function*, Elsevier, N.Y., pp. 646.

Danks H. V. (1987) *Insect Dormancy: An Ecological Perspective*, Biological Survey Canada, Ottawa.

Daniel W. W. (1999) *Biostatistics: A Foundation for Analysis in the Health Sciences*, John Wiley & Sons, New York, pp. 755.

De Clercq P. and Degheele D. (1992) Development and survival of *Podisus maculiventris* (Say) and *Podisus sagitta* (Fab.) (Heteroptera: Pentatomidae) at various constant temperature. Canadian Entomologist, 124: 125–133.

De Clercq P. and Degheele D. (1993) Cold storage of the predatory bugs *Podisus maculiventris* (Say) and *Podisus sagitta* (Fabricius) (Heteroptera: Pentatomidae). Parasitica, 49(1-2): 27–41.

Isenhour D. J. and Yeargan K. V. (1981) Effect of temperature on the development of *Orius insidiosus*, with notes on laboratory rearing. Annals of Entomological Society of America, 74: 114–116.

Morimoto S., Imamura T., Visarathanonth P. and Miyanoshita A. (2007) Effects of temperature on the development and reproduction of the predatory bug *Joppeicus paradoxus* Puton (Hemiptera: Joppeicidae) reared on *Tribolium confusum* eggs. Biological Control, 40(1): 136–141.

Nishi A. and Takahashi K. (2002) Effects of temperature on oviposition and development of *Amphibolus venator* (Klug) (Hemiptera: Reduviidae), a predator of stored product insects. Applied Entomology and Zoology, 37(3): 415–418.

Parker A. H. (1972) The predatory and sexual behaviour of *Phonoctonus fasciatus* (P.de B) and *P. subimpictus* Stål (Hemiptera: Reduviidae). Bulletin of Entomological Research, 62: 139–150.

Rahman M. M., Islam W. and Ahmed K. N. (2007) Effect of temperature on the predator, *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) feeding on *Cryptolestes pusillus* (Schon.) (Coleoptera: Cucujidae). Journal of bio-sciences, 15: 41–46.

Ruberson J. R., King T. J. and Elkassabany N. (1998) Over-wintering and the diapause syndrome of predatory Heteroptera. In: *Predatory Heteroptera; their Ecology and Use in Biological Control*, Coll M. and Ruberson J. R. (Eds). Entomological Society of America, Thomas Say Publication, Entomological Society of America, Lanham, MD, pp. 89–130.

Usharani P. (1992) Temperature-induced effects on predation and growth of *Eocanthecona furcellata* (Wolff) (Pentatomidae: Heteroptera). Journal of Biological Control, 6(2): 72–76.

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Jalaja M., Muraleedharan D. and Prabhu V. K. K. (1973) Effect of extirpation of median neurosecretory cells on reproduction in the female red cotton bug, *Dysdercus cingulatus*. Journal of Insect Physiology, 19(1): 29–36.

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Wigglesworth V. B. (1964) The hormonal regulation of growth and reproduction in insects. In: *Advances in Insect Physiology*, vol. 2 (Eds. Beament J. W. L., Treherne J. E. and Wigglesworth V. B), Academic Press, London, pp 247–335.

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manuscript will be scrutinized again by an Editorial team (and by expert referees, if needed) before final acceptance. On final acceptance, the author will be asked to submit an electronic version of the manuscript. Proof will be sent to the corresponding author. It should be checked and returned within three days of receipt. The journal reserves the right to proceed with publication if corrections are not communicated promptly.

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